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THE AMERICAN REVIEW OF TUBERCULOSIS

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MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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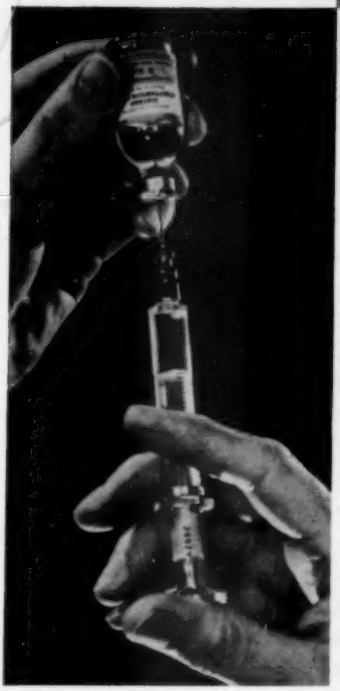

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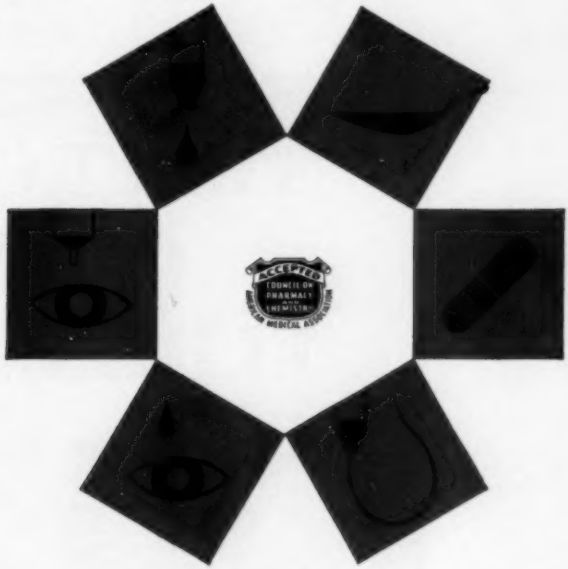

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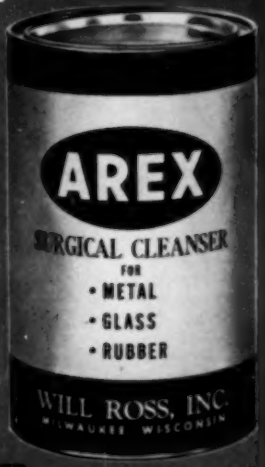

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THE AMERICAN REVIEW
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VOLUME 64 *October 1951* NUMBER 4

TUBERCULOMAS OF THE MEDIASTINUM[1,2]

CLIFFORD F. STOREY AND HAROLD A. LYONS

(Received for publication May 4, 1951)

The extremely small number of mediastinal tuberculomas reported in the literature might easily lead to the belief that the lesion is one of great rarity. The writers have recently observed 6 cases of mediastinal tuberculoma, however, 5 of them proved at operation, and it is our conviction that these masses of tuberculous origin are much more common than might be surmised on the basis of the few cases reported. Not only is it believed that specific granulomas of this type occurring in the mediastinum are not particularly unusual, but it is also believed that sufficient accumulated experience will permit a correct preoperative diagnosis to be made with reasonable certainty in an appreciable percentage of cases. These considerations are subjected to review in the present report which is based on the experiences with the 6 previously unreported instances of mediastinal tuberculoma.

CASE REPORTS

Case 1. M. A. O'B. (Reg. No. 94983). The patient, a 24-year-old sailor, was admitted to the U. S. Naval Hospital, St. Albans, N. Y., on May 13, 1947 because of a mediastinal mass discovered on routine roentgenographic examination of the chest. A chest roentgenogram taken five years previously had been reported as negative, but this film was not available for review. The patient had no complaints and the general physical examination revealed no significant abnormalities. The past history revealed no relevant facts and his family history was likewise irrelevant to consideration of the present illness. There was no tuberculosis in the patient's family and he had had no known intimate exposure to the disease.

Roentgenographic studies: Chest roentgenograms (figure 1) showed a right superior mediastinal mass which measured approximately 5 cm. in diameter and was situated above the right hilum. In the lateral view the lesion was seen to be located in the mid-mediastinum. Fluoroscopy showed that it did not move with the lung on respiration, and barium swallow demonstrated that it did not ascend on deglutition. There appeared to be no impingement on either the esophagus or trachea. Angiocardiography established the fact that the lesion was not vascular in nature. Only slight compression of the superior vena cava at its junction

[1] From the Department of Thoracic Surgery, U. S. Naval Hospital, St. Albans, New York.

[2] The opinions or assertions contained herein are those of the writers and are not to be construed as official or reflecting the views of the Navy Department or of the naval service at large.

with the azygos vein was shown. Tomograms failed to reveal evidence of calcium deposits in the mass and established that the lesion was solid rather than cavernous.

The tuberculin reaction was positive in the weakest dilution. Other skin tests were negative. All laboratory tests yielded values which were within normal limits. Bronchial aspirates were negative for tubercle bacilli.

Operation: On August 6, 1947 a right posterolateral thoracotomy was performed (by Dr. J. Maxwell Chamberlain). The chest was entered through the bed of the resected fourth rib. A round, circumscribed, firm, tensely cystic mass was found projecting from the right superior mediastinum. The inferior surface of the mass occupied the crotch formed by the junction of the azygos vein and the superior vena cava. Each of these veins was somewhat compressed by the mass. The posteromedial wall of the mass lay in juxtaposition to the anterolateral wall of the trachea and inferiorly it was in close proximity to the right main

FIG. 1. *Case 1.* (M. A. O'B.). Posterior-anterior chest roentgenogram showing the smoothly outlined right superior mediastinal mass. A lateral film showed it to be situated in the mid-mediastinum.

stem bronchus. Both the right vagus and phrenic nerves coursed over the mass. Although it was definitely encapsulated, the capsule was densely adherent to the adjacent structures and could be freed from them only by tedious dissection. Even the overlying pleura was stripped with considerable difficulty. The tumor was removed intact without damage to the mediastinal structures.

Pathologic report: On macroscopic examination, "grossly, the specimen was an ovoid cystic mass 5.0 by 3.2 by 2.0 cm. The outer surface presented a granular, grayish-white to violaceous appearance. The cystic contents were of a caseous nature."

Microscopic examination: On microscopic examination (figure 2), "the lumen was filled with calcified caseous debris and the wall consisted of dense hyalinizing fibrous connective tissue and chronic granulomatous tissue characterized by epithelioid tubercles with occasional Langhans' type of giant cells, scattered lymphocytes, plasma cells, and macrophages. No lymph node could be identified. Ziehl-Neelsen stains were positive for acid-fast bacilli."

Diagnosis: Tuberculoma, mediastinal.

The patient's postoperative course was uncomplicated. Serial chest films taken following operation showed no parenchymal or mediastinal abnormality (figure 3) and he was returned to full duty. He has remained well.

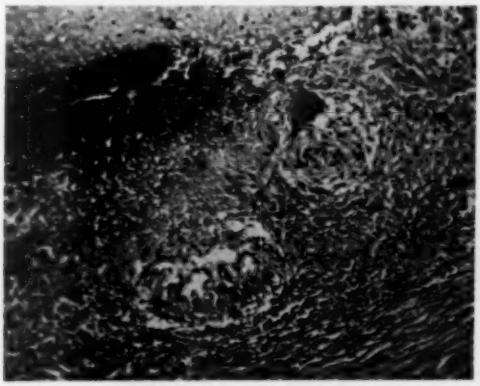


FIG. 2. *Case 1.* Miscroscopic section through area adjacent to cyst wall showing calcified caseous debris (darkly stained area) surrounded by tubercles. × 220.

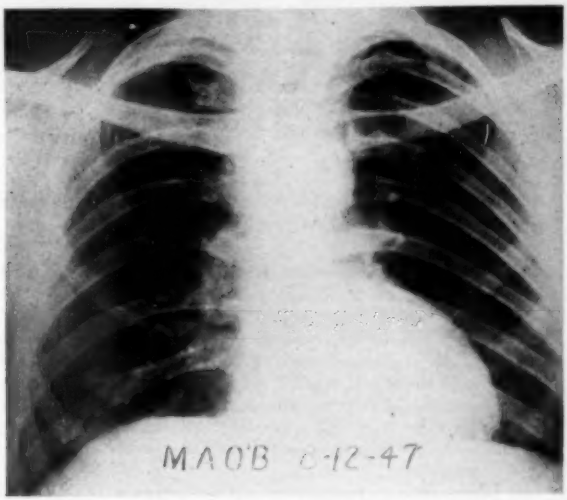
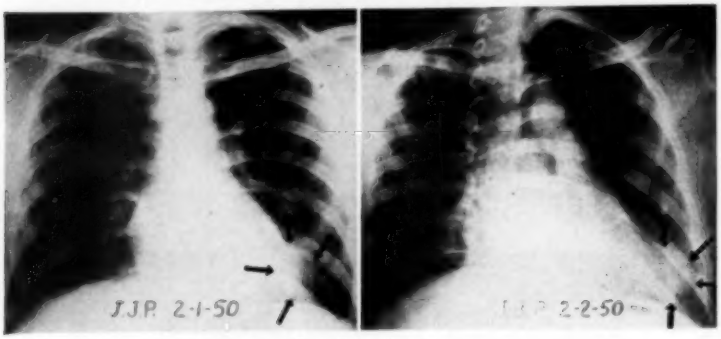


FIG. 3. *Case 1.* Postoperative chest roentgenogram. The lung fields have remained clear.

Case 2. J. J. P. The patient, a 20-year-old white male, was admitted to the U. S. Naval Hospital, St. Albans, N. Y. on February 16, 1950 because of a mass situated along the left cardiac border which was discovered on routine chest roentgenography. He had no symptoms. His past history revealed that in 1947 and again in 1949 he had complained of precordial pain on vigorous exercise, several small hemoptyses, night sweats, and approxi-

mately 25 pounds' loss in weight. On each occasion he was hospitalized elsewhere, where intensive studies failed to result in a definite diagnosis. The lesion for which he was admitted to this hospital was seen on those occasions. Among the entities considered at the other hospitals were echinococcus disease, pericardial cyst, and atelectasis of the lingula. His family history was irrelevant. There had been no known exposure to tuberculosis. General physical examination was entirely negative.

Roentgenographic studies: Roentgenographic examination of the chest (figures 4 and 5) showed a soft tissue density closely associated with the apex of the heart and measuring roughly 4 cm. in diameter, with a broad base confluent with the apex of the heart. The density of this mass was less than that of the corresponding heart shadow, suggesting the possibility that it contained fat. The lung fields were clear. The 1947 films were not available for comparison. Foreign films dated November 30, 1949 showed the lesion to be approximately the same size as at the time of his admission at St. Albans. The mass could not be defined on



Case 2 (J. J. P.)

FIG. 4. (Left) Posterior-anterior chest film showing the oval area of increased density partly overlying and projecting to the left from the left border of the heart near the cardiophrenic angle.

FIG. 5. (Right) Left posterior oblique chest roentgenogram delineates the mass to somewhat better advantage than the standard posterior-anterior projection.

the lateral film. On fluoroscopy it was seen to be situated anteriorly. It pulsated synchronously with the heart beat, but the pulsations were thought to be transmitted rather than expansile. The mass appeared to change slightly in contour with respiration but did not move with the lung. Angiocardiography showed good opacification of the heart chambers and major blood vessels following introduction of 50 per cent Diodrast into the right brachial system, but the abnormal shadow at the left cardiac border did not become opaque.

Bronchoscopy was negative. The tuberculin skin reaction was negative. Ballistocardiograms and electrocardiograms were within the limits of normal. Four sputum specimens and one bronchial aspirate were negative on culture for tubercle bacilli. The preoperative impression was *pericardial cyst.*

Operation: Left posterolateral thoracotomy was performed on March 21, 1950 (by Dr. T. C. Ryan). The chest was entered through an intercostal incision in the fifth interspace. The visceral and parietal pleurae were completely fused, but the adhesions were thin and filmy and separated easily. The tumor lay in the anterior cardiophrenic angle between the sternum and the pericardium. The mass was firm but not hard. Portions of the lesion freed easily from the pericardium to which it was adherent. It also separated readily from most of the sternum and a small fat pad with which it was intimately associated. However, at

one site it was densely adherent to the overlying sternum, and on sharp division of the adhesions at this point the mass was entered and a small quantity of thick yellow purulent-appearing material escaped. Sharp dissection was also required to free one small area from the

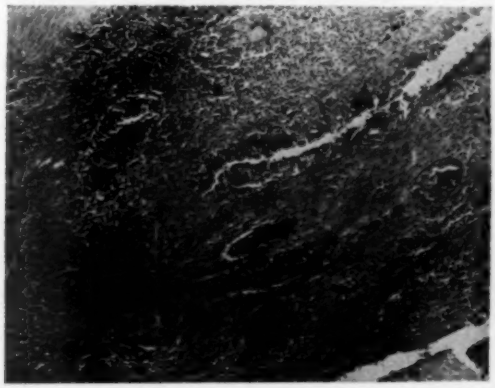


FIG. 6. *Case 2.* Microscopic section showing lymph node architecture largely replaced by tuberculous granulation tissue with caseous necrosis in the center. × 100

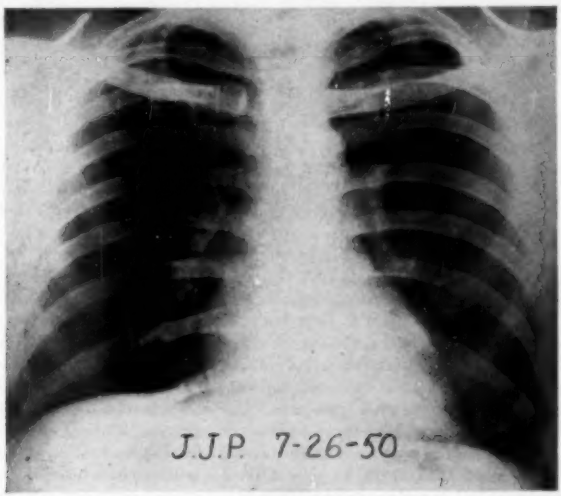


FIG. 7. *Case 2.* The mass is no longer present. The pulmonary parenchyma has remained clear.

pericardium. The mass was oval in shape and approximately 4 cm. in the short diameter and 6 cm. in the long axis. The mass looked and felt cystic. The postoperative impression was *dermoid cyst.*

Pathologic report: On macroscopic examination, "grossly, the specimen was a partly cystic mass 7.0 by 6.0 cm. The outer surface presented a smooth, mottled grayish-red appearance. The cut surface was of similar color but more granular. An irregular cystic space was present in the center and was filled with yellowish-green purulent exudate. The cyst lining consisted of friable, grayish granular material."

Microscopic examination: On microscopic examination (figure 6), "there was a severe, chronic granulomatous lymphadenitis with replacement of the normal architecture of the lymph node by epithelioid tubercles. The latter presented caseous centers, numerous Langhans' type of giant cells, and lymphocytic cuffs. The tubercles became confluent in some areas, frequently with purulent abscess formation. Fibrous scarring was also present. Ziehl-Neelsen stains were negative for acid-fast bacilli."

Diagnosis: Granulomatous and suppurative inflammation, most likely tuberculosis. The gross and microscopic findings suggest an encapsulated mediastinal abscess of tuberculous origin.

Postoperatively the patient developed a sanguineous pleural effusion which was loculated laterally in the lower chest. Several thoracenteses were performed but the fluid, consisting of a large content of clotted blood, was aspirated with difficulty. It absorbed rather rapidly, however, and thereafter the chest was clear on physical and roentgenographic examination. A 42-day course of streptomycin, 1 gm. daily, and para-aminosalicylic acid (PAS), 12 gm. daily, was given. The patient was maintained on a regimen of bed rest with increasing intervals out of bed for a total period of six months. His chest roentgenograms remained normal (figure 7); he was afebrile, asymptomatic, and gained weight. Six postoperative sputum specimens were negative for tubercle bacilli on smear and culture. At the end of six months he was returned to full duty.

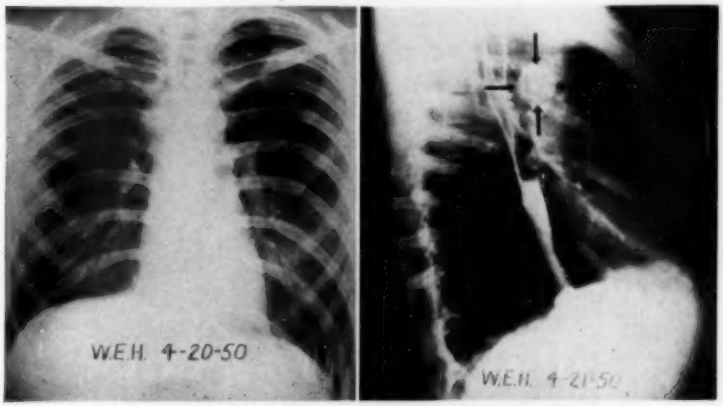
Case 3. W. E. H. The patient, a 25-year-old white man, was admitted to the U. S. Naval Hospital, St. Albans, N. Y., on June 14, 1950 because of a mediastinal mass discovered on routine chest roentgenography. He had no complaints, and physical examination revealed no abnormal findings. Prior to entering this hospital he had been studied in another hospital for two months without establishment of a diagnosis. Bronchoscopy, bronchography, sputum cultures, and a gastrointestinal series at that activity had shown normal findings. The patient's past medical history and family history revealed no relevant facts. For a period of sixteen months, beginning in 1947, the patient had associated with a young woman who was subsequently discovered to have active pulmonary tuberculosis. Review of a chest film taken in 1948 showed the lesion to have been present at that time also but it had subsequently increased in size.

Roentgenographic studies: Stereoscopic frontal (figure 8) and right lateral chest roentgenograms showed a superior mediastinal mass projecting to the right at the level of the junction of the azygos vein and superior vena cava. The mass was oval in shape, approximately 4 cm. in diameter, smoothly outlined, and sharply marginated. A faint rim of calcification was seen at the periphery of the mass. In the lateral view (figure 9) it was seen to lie in the midplane of the chest. The inferior margin of the mass lay at the level of the right main stem bronchus, and its posteromedial wall lay along the anterolateral wall of the trachea. Fluoroscopy confirmed the location of the mass as seen on the films and showed that it was extrapulmonary in location as it did not move with the lung on respiration. The lung fields were clear. Angiocardiography showed that the lesion was not vascular. Barium swallow (figure 9) showed no abnormality of the esophagus.

Bronchoscopy was entirely negative. There was no evidence of extrinsic pressure on either the trachea or the right main stem bronchus. The Friedman test (done to rule out

the possibility that the mass represented a metastasis from a primary embryonal carcinoma) was negative, as were the coccidioidin and histoplasmin skin tests. The tuberculin skin reaction was definitely positive in 1:10,000 dilution. Four sputum specimens and one bronchial aspirate were negative for tubercle bacilli on smear and culture. The preoperative impression was *mediastinal tuberculoma*.

Operation: A right posterolateral thoracotomy was performed on July 25, 1950 (by C. F. S.). The chest was entered through an intercostal incision placed in the fifth interspace. A free pleural space was encountered except for an area approximately 3 cm. in diameter on the mediastinal surface of the inferior portion of the right upper lobe which was firmly adherent to the underlying tumor mass. When the adhesions had been severed and the lung retracted, a mass 4 cm. in diameter was seen lying in the crotch between the



Case 3 (W. E. H.)

FIG. 8. (Left) Posterior-anterior chest roentgenogram showing the sharply circumscribed mass of homogeneous density projecting to the right from the mediastinum just above the hilum.

FIG. 9. (Right) Lateral roentgenogram with barium swallow showing the mass to be situated slightly anterior to the mid-plane of the chest. Note spherical shape and sharp demarcation. The esophagus is normal.

azygos vein and the superior vena cava. Inferiorly it extended to the level of the right main stem bronchus to which it was adherent, its medial wall was densely adherent to the superior vena cava, and posteromedially it lay against the anterolateral wall of the trachea. The right phrenic and vagus nerves crossed the tumor capsule as they descended in the chest. The mass was firmly adherent to the adjacent structures and was separated with considerable difficulty by a combination of sharp and blunt dissection. The chronic inflammatory adhesions between the mass and the cava were especially tenacious and only by slow and tedious dissection could the mass be released from the vein without tearing it. The mass was finally freed and removed intact.

Pathologic report: On macroscopic examination, "grossly, the specimen was a spherical, cystic mass 3.0 cm. in diameter. The outer surface presented a relatively smooth, grayish-white appearance except for an occasional fibrous tag. The cut surface was of similar color but more granular. A cystic cavity filled with gritty caseous material was lined by granular, trabeculated tissue of friable consistency."

Microscopic examination: On microscopic examination (figure 10), "the wall consisted of dense fibrous connective tissue with groups of epithelioid cells which formed poorly defined tubercles. Occasional Langhans' type of giant cells, lymphocytes, and a few plasma cells were also present. No lymph node could be identified. Ziehl-Neelsen stains were positive for acid-fast bacilli."

Diagnosis: Tuberculoma, mediastinal.

The patient's postoperative course was uneventful. His chest roentgenograms were normal (figure 11) and remained so. He was given streptomycin and PAS for 42 days (1 and 12 gm. daily, respectively). He was maintained on a bed-rest regimen with progressively increasing intervals out of bed for a period of six months. Six postoperative gastric lavage concentrates were negative for tubercle bacilli on culture. He remained afebrile, asymptomatic, and gained weight. Six months following operation he was returned to full duty.

FIG. 10. *Case 3.* Microscopic section showing cyst wall surrounded by a layer of epithelioid cells without good tubercle formation. × 220.

Case 4. D. P. G. This patient, a 20-year-old white male, was admitted to the U. S. Naval Hospital, St. Albans, N. Y. on June 14, 1950 complaining of headache, malaise, and anorexia of nine days' duration. He had had a cough for the four days preceding entry in the hospital and was raising small amounts of greenish, blackish sputum. For several weeks prior to admission he had been working in the interior of ships on a cleaning detail which entailed considerable exposure to ferrous dusts (rust). His past medical history revealed nothing of significance. The family history was irrelevant. There was no known tuberculosis in his family. During a previous hospitalization for an unrelated illness one year prior to his admission here, he had worked in the wards for several weeks and may have been exposed to tuberculosis at that time, although no definite exposure could be established. Photofluorograms of the chest taken September 19, 1947 and December 13, 1949 were reported as normal. General physical examination revealed no significant abnormalities and, specifically, there were no abnormal findings with reference to the chest. The tonsils were chronically diseased and he was found to have epidermophytosis of both feet and tinea cruris (ringworm). Routine laboratory examinations revealed normal findings. A serologic test for syphilis (Kahn) was negative. The tuberculin, coccidiodin, and histoplasmin skin tests were negative. The tuberculin reaction was tested in three dilutions but remained negative even to the 1:500

dilution. Bronchoscopy showed a small amount of purulent secretions in the right lower lobe bronchus which did not reappear on cough following aspiration. The middle lobe bronchial orifice appeared unusually prominent as if displaced somewhat posteriorly. Two sputum specimens and one bronchial aspirate were negative for tubercle bacilli on smear and culture.

Roentgenographic studies: Roentgenograms of the chest (figure 12) showed an oval-shaped mass 3.5 cm. in diameter projecting to the right from the superior mediastinum at the level of the junction of the azygos vein with the superior vena cava. In the lateral view it was seen that the mass lay slightly anterior to the midplane of the chest (figure 13). The mass was sharply circumscribed, smoothly marginated, and of homogenous density. A tracheogram (figure 14) showed the mass to lie immediately adjacent to the right antero-

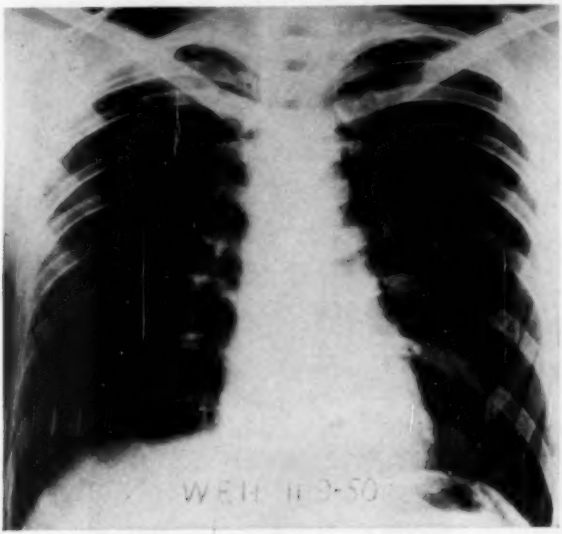
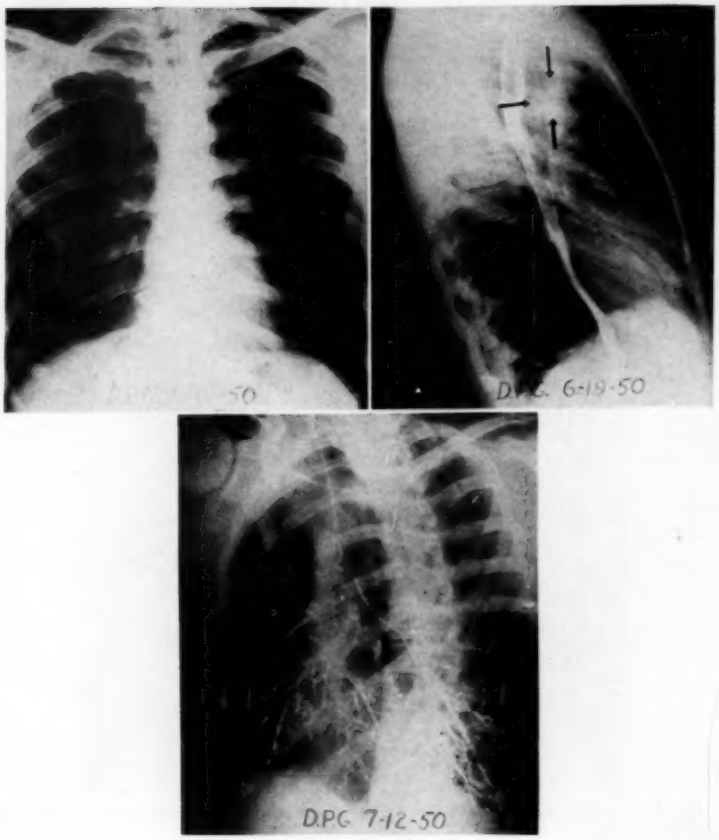


FIG. 11. *Case 3.* Postoperative chest film. The mass has been removed. The lung fields appear normal.

lateral wall of the trachea but there was no tracheal distortion or indentation. Bronchography showed no evidence of bronchiectasis. Barium swallow (figure 13) showed no deviation of the esophagus. Fluoroscopy confirmed the position of the mass as seen on the films and showed that the mass was extrapulmonary in location for it did not move with the lung on respiration. Angiocardiography showed that the lesion was not vascular in nature. The preoperative impression was *mediastinal tuberculoma.*

Operation: Right posterolateral thoracotomy was performed on July 27, 1950 (by C. F. S.). The chest was entered by an incision in the fourth intercostal space. A free pleural space was encountered except for a small area on the mediastinal surface of the inferior portion of the right upper lobe which was densely adherent to the tumor adjacent to it. Upon severing the adhesions and mobilizing the lung, the mass was seen to be approximately 4 cm. in diameter. It lay in the crotch formed by the junction of the azygos vein and the superior vena cava. Inferiorly it abutted upon the superior margin of the proximal portion of the right main stem bronchus. Its posteromedial surface lay against the trachea. The right



Case 4 (D. P. G.)

FIG. 12. (Left) Posterior-anterior chest roentgenogram showing a mass roentgenographically identical with that seen in Case 3 (see figure 8).

FIG. 13. (Right) Lateral projection with barium swallow. In this view also, the mass also duplicates that seen in Case 3 (see figure 9).

FIG. 14. (Bottom) Left anterior oblique bronchogram showing the right superior mediastinal mass to lie in close juxtaposition to the trachea and to rest inferiorly on the right main stem bronchus.

phrenic and vagus nerves coursed over the mass as they descended in the chest. On palpation the mass was found to be of firm, semi-solid consistency and it did not move freely. The tumor could not be enucleated easily as it was densely adherent to the structures adjacent to which it lay, and slow and tedious combined blunt and sharp dissection was required to effect its release. This was accomplished without mishap, however, and the mass was removed intact. Careful examination at operation revealed no enlarged mediastinal

nodes. The patient's postoperative course was uncomplicated except for the formation of a sterile pleural effusion which required thoracentesis on three occasions.

Pathologic report: On macroscopic examination "grossly, the specimen was an ovoid, cystic mass 3.0 cm. in diameter. The outer surface presented a finely granular grayish-brown appearance with a few tags of fibrous tissue. The cyst wall was lined by granular, brownish-gray, friable tissue and the lumen contained gritty caseous material. The appearance of the cut surface of the solid tissue was similar to that of the outer surface."

Microscopic examination: On microscopic examination (figure 15), "the wall was made up of dense, hyalinizing, fibrous connective tissue containing calcified, caseous debris. Only scattered lymphocytes, macrophages, and plasma cells were seen with no evidence of specific chronic granulomatous inflammation. No lymph node could be identified. Smears and cultures taken from the cyst lining were negative for tubercle bacilli."

Diagnosis: Consistent with tuberculoma, inactive.

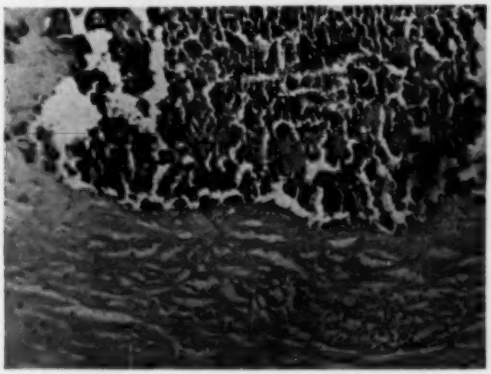


FIG. 15. *Case 4.* Microscopic section showing hyalinized connective tissue wall containing only scattered inflammatory cells. Calcified debris is seen in the upper portion of the photograph. × 220

Postoperatively, the patient was arbitrarily given streptomycin, 1 gm., and PAS, 12 gm., daily for ninety days. He was kept on bed rest for six months. He remained afebrile and asymptomatic. Six additional sputum cultures were negative for tubercle bacilli. Chest roentgenograms (figure 16) were normal postoperatively and remained so. The symptoms for which the patient was admitted subsided soon after entry and did not recur. He was returned to full duty.

Case 5. C. M. The patient, a Negro male 30 years of age, was admitted to the U. S. Naval Hospital, St. Albans, N. Y. on August 16, 1950 complaining of pain of two weeks' duration in the right shoulder, right arm, and chest. He had felt well until approximately two weeks before admission when he developed progressive pain in the regions mentioned. It became so severe that he could not raise his arm. He also complained of pain substernally on swallowing which was referred to the right side of the neck. Similar pain was caused by deep inspiration. The patient's past history was unrevealing and there had been no intimate exposure to tuberculosis. His family history revealed no relevant facts. The general physical examination disclosed no significant abnormalities.

Roentgenographic studies: Roentgenographic examination of the chest (figure 17) was

reported as follows: "Studies of the chest disclosed the presence of a homogeneous shadow of increased density in the right paratracheal region. The lateral margin of this density is sharply defined and the medial border is lost in the shadow of the mediastinum. The trachea is in the midline and shows no evidence of compression or displacement. The lung fields show no evidence of parenchymal disease. On stereoscopic studies the density in question is seen to lie within the mid-portion of the superior mediastinum. This density is considered to represent a mass in the superior mediastinum. The most likely possibility is that it represents a disease of the lymph nodes. Of these, one might include sarcoid, Hodgkin's disease, and tuberculosis. Among the other diagnostic possibilities one might consider for a mass in

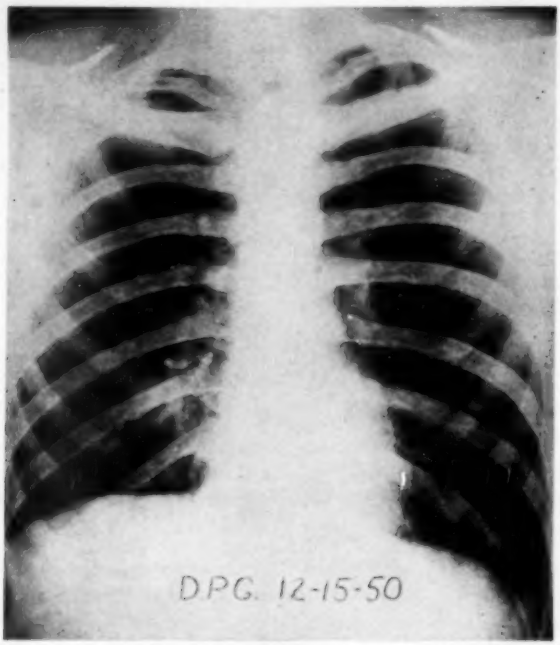


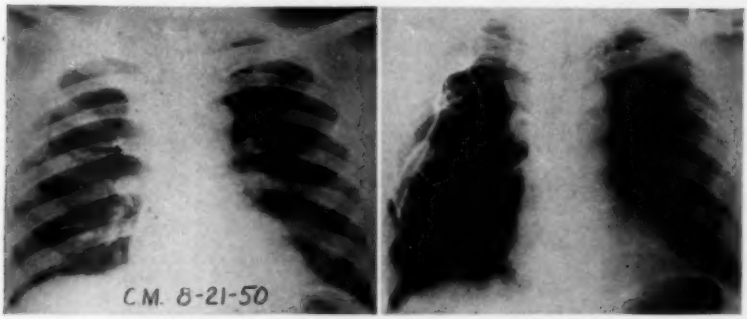
FIG. 16. *Case 4.* Postoperative chest roentgenogram. There is no evidence of pleural or parenchymal involvement. The mass is no longer in evidence.

this region are a retrosternal thyroid and an aneurysm of the aorta. These are believed to be ruled out by virtue of the normal appearance of the trachea and the fact that the mass in question has a lobular appearance and is in the vicinity of the paratracheal nodes. There are other less common diagnostic possibilities, such as hematoma, metastatic tumor, and various other tumors of the lung and mediastinum but these cannot be diagnosed without histological study." Lateral chest film had shown the mass to be centered in the mid-plane of the chest.

Bronchoscopy showed marked evidence of extrinsic pressure on the right lateral wall of the middle and lower thirds of the trachea, the wall being displaced medially. The main carina was blunted and rotated into the "10 o'clock–4 o'clock" position. There were a few scattered flecks of whitish secretions in the right bronchial tree which did not reappear on

cough after aspiration. A biopsy was taken from the right lateral wall of the lower trachea even though the mucosa appeared grossly normal. On microscopic study the biopsy specimen showed only chronic inflammation. Bronchial aspirate was negative for malignant cells on Papanicolaou stain.

The tuberculin reaction to the "first-strength" dose was definitely positive. Coccidiodin and histoplasmin tests were negative. One bronchial aspirate and four sputum specimens were negative for tubercle bacilli on smear and culture. An enlarged node was excised from the right axilla and on histologic study it showed only chronic lymphadenitis. An angiocardiogram made following introduction of dye into the right brachial system showed complete obstruction of the right subclavian vein near the origin of the right innominate vein (figure 18). The collateral venous circulation was demonstrated but no dye reached the cardiac chambers during the examination. The examination was repeated by injecting the contrast media into the left brachial vein. This showed considerable narrowing of the superior vena cava at and above the level of the hilum. It was believed that this was caused



Case 5 (C. M.)

FIG. 17. (Left) Posterior-anterior chest film showing the right superior mediastinal mass. It is somewhat more elongated than the "typical" lesion.

FIG. 18. (Right) Preoperative angiocardiogram showing obstruction of the right subclavian vein. This finding raised a strong presumption of malignancy. Note collateral circulation.

by extrinsic pressure of the mass above the right hilum. Ballistocardiograms and electrocardiograms were normal. A bone marrow study showed no abnormalities, and peripheral blood studies were normal. A serologic test for syphilis (Kahn) was negative. Esophagram was normal. Fluoroscopy confirmed the presence of the mass and showed that it lay outside the lung parenchyma. Venous pressure in the right arm was 360 mm. of water and, after finger exercises, was 420 mm. of water. In the left arm the pressure was 200 mm. of water and was unchanged after finger exercises. The preoperative impression was *probable lymphoblastoma*.

Operation: Right posterolateral thoracotomy was performed on September 19, 1950 (by C. F. S.). The fifth rib was resected. A free pleural space was encountered. The lung was grossly normal. A fairly large, firm-to-hard, fleshy-appearing, poorly delimited mass was seen and felt in the right upper mid-mediastinum. It extended from a point above the superior thoracic aperture, through the inlet, and down alongside the great veins to the azygos. There was a prolongation of the mass below the transverse portion of the azygos vein extending to the level of the inferior pulmonary vein. The mass also extended outward along the lung root, surrounding, at least in part, the right main stem bronchus. In the anterior-posterior direction the mass covered the esophagus posteriorly and was in-

distinguishable from it. The mass completely obscured the right side of the trachea and was fused to the superior vena cava. Tentative exploratory efforts were made to establish a cleavage plane between the mass and the adjacent structures, but so intimately was it fused to them by dense chronic inflammatory tissues that no cleavage could be found. It became apparent that removal of the mass would be exceedingly dangerous from the standpoint of injury to the important structures to which it was adherent. It is worthy of note that, although irregularly sausage-shaped, somewhat lobulated, and possessing pseudopod-like prolongations as mentioned, this appeared to be one mass rather than a conglomeration of masses or a group of fused nodes. In the conviction that, if the mass represented a malignant tumor, a curative resection was impossible; and, if benign, an attempt at resection would be more hazardous than leaving the tumor *in situ*, a generous biopsy was taken from the most prominent part of the tumor above the azygos vein. When the biopsy was taken, a large degenerating cavernous mass was entered and a thick, creamy-yellow purulent material escaped. The pus was aspirated and additional bits of the wall of the cavity were excised.

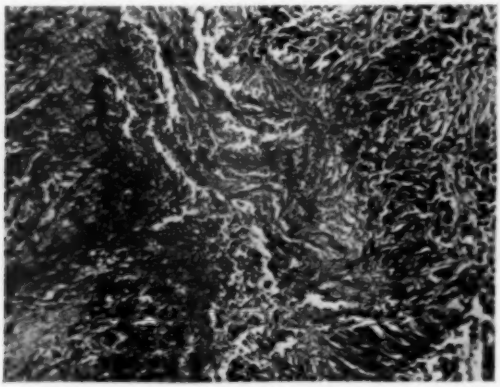


FIG. 19. *Case 5.* Microscopic section showing a central area of caseation surrounded by radiating epithelioid cells, fibroblasts, and occasional Langhans' type of giant cells. × 220

Examination of frozen sections of the tissue was reported as showing a chronic granuloma consistent with tuberculosis. As much of the tumor was excised as could reasonably be accomplished within the limits of safety, hoping thereby to relieve the venous obstruction. Two grams of streptomycin and one million units of penicillin were instilled into the pleural space and the chest was closed.

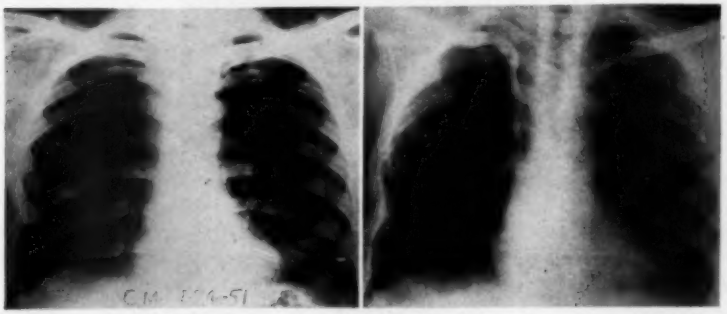
Pathologic report: On macroscopic examination "grossly, the specimen consisted of a few irregular fragments of grayish-brown tissue of firm to friable consistency."

Microscopic examination: On microscopic examination (figure 19) "there was chronic granulomatous lymphadenitis. Well-formed epithelioid tubercles with central caseation and Langhans' type of giant cells were present. Other sections revealed more severe caseation with beginning cyst formation. Some fibrosis and fibroblastic proliferation was seen. Ziehl-Neelsen stains were negative for tubercle bacilli."

Diagnosis: Consistent with caseous tuberculosis of mediastinal lymph nodes.

The patient's postoperative course has been entirely uneventful. He has remained afebrile. Serial roentgenograms of the chest (figure 20) have shown the lung fields to be clear. They have also shown a slow but progressive diminution

in size of the mediastinal mass. The patient was given 1 gm. of streptomycin and 12 gm. of PAS daily for 120 days postoperatively. Three postoperative sputum specimens have been negative for tubercle bacilli on smear and culture. The patient has been maintained on a bed-rest regimen with gradually increasing time out of bed. Two postoperative angiocardiograms (figure 21), in which the dye was injected into the right brachial vein on one examination and into the left on the other, demonstrated almost complete relief of the obstruction shown on the preoperative studies. There remained only slight compression of the inferior vena cava. All of the symptoms of which the patient complained have subsided. He has remained entirely free of pain. It is anticipated that at the end of six months this patient will be returned to full duty.



Case 5

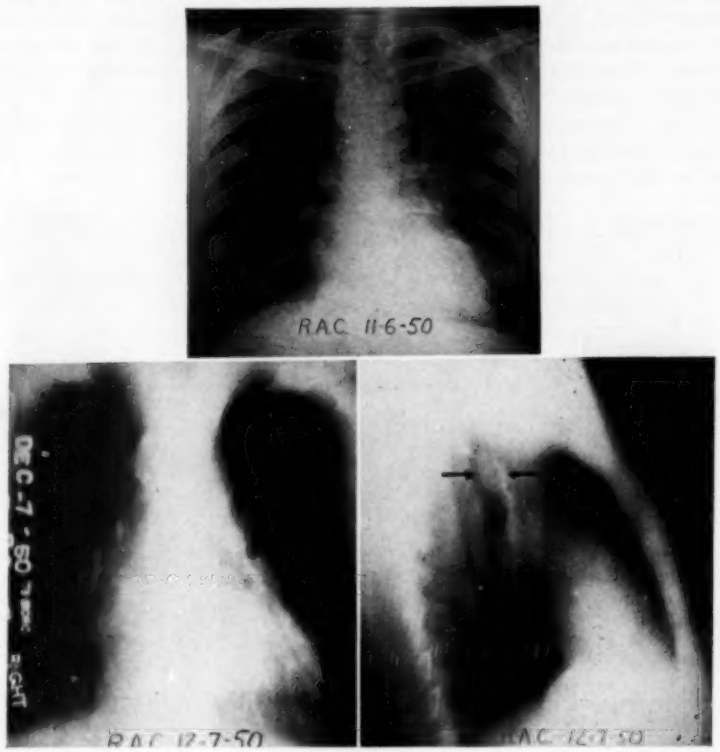
FIG. 20. (Left) Postoperative posterior-anterior chest film showing definite diminution in the size of the mass (Compare with figure 17).

FIG. 21. (Right) Postoperative angiocardiogram. Note complete relief of the subclavian vein obstruction. The superior vena cava is well filled although somewhat compressed by the residual mass. Note relative disappearance of the extensive collateral network leading to the azygos system seen in figure 18.

Case 6. R. A. C. The patient, a 30-year-old white man, was admitted to the U. S. Naval Hospital, St. Albans, N. Y. on November 2, 1950 with the tentative diagnosis of *neurofibroma, mediastinum*, because of a mass discovered on routine chest roentgenograms taken at the time of his recall to active duty in the Navy. The patient had no complaints, but on questioning admitted a slight chronic cough which had been present without change for several years. His past medical history and family history were unrevealing and there had been no known exposure to tuberculosis. Physical examination showed a healthy, robust, adult male with no abnormalities. A serologic test for syphilis (Kahn) was negative. The tuberculin reaction was one plus to the "second strength." The histoplasmin and coccidiodin tests were negative. Six gastric-washing concentrates were negative for tubercle bacilli on culture. Other laboratory examinations revealed values which were within normal limits.

Roentgenographic studies: Roentgenographic examination of the chest (figure 22) showed an oval mass of apparently homogeneous density situated in the superior mediastinum at the level of the junction of the azygos vein and superior vena cava. The right lateral film showed the mass to lie in the mid-plane of the chest. The mass was sharply demarcated and of smooth outline. No calcium could be seen in the standard films. Fluoroscopy confirmed the presence of the mass and showed it to be extrapulmonary in location as it did not move with the lung on respiration. Posterior-anterior (figure 23) and lateral (figure 24) tomo-

grams showed numerous calcium deposits scattered throughout the mass. Its inferior margin was seen to lie in juxtaposition to the right main stem bronchus, and its posteromedial wall lay adjacent to the anterolateral wall of the trachea. An area of pleural calcification on the right was also demonstrated. Bronchoscopy showed blunting of the main carina. The



Case 6 (R. A. C.)

FIG. 22. (Top) Posterior-anterior roentgenogram of the chest showing the right superior mediastinal mass almost identical with that seen in Cases 3 and 4. In this standard film the calcification within this mass is not apparent.

FIG. 23. (Lower left) Posterior-anterior tomogram at the 10-centimeter level showing definite calcification within the mass.

FIG. 24. (Lower right) Lateral tomogram at the 10-centimeter level. The mid-mediastinal location of the lesion is established. Calcification can again be seen.

posterior tracheal wall at the carina appeared to be pushed forward. The carina was rotated slightly in a clockwise direction. These findings were interpreted as due to extrinsic pressure (subcarinal nodes). The bronchoscopic findings were otherwise normal. Angiocardiography was not done. Barium swallow showed a normal esophagus.

Papanicolaou and acid-fast stains of the bronchial aspirate were negative for tumor cells or acid-fast bacilli. The erythrocyte sedimentation rate remained consistently normal.

Comment: Previous experience with lesions almost identical in size, shape, and position aroused a strong suspicion that this lesion was a tuberculoma. The findings at bronchoscopy were compatible with that diagnosis. The tuberculin test was positive as anticipated. Demonstration of marked calcification within the mass by tomography was accepted as reasonably convincing evidence that it was a tuberculoma. In view of six concentrated gastric washings negative for tubercle bacilli on culture and one negative bronchial aspirate for tubercle bacilli on smear and culture, a normal erythrocyte sedimentation rate, a completely afebrile hospital course, and the absence of any symptoms, it was believed that a clinical diagnosis of tuberculoma, mediastinal, was justified. The patient was returned to duty. He will be followed by periodic chest roentgenograms to check for possible increase in size or change in character of the mass.

DISCUSSION

The general principle that all isolated tumors of the mediastinum should be surgically extirpated has been widely accepted by those interested in surgery of the thorax. There are compelling reasons why such a policy has been adopted. Many of these lesions will prove to be malignant. Others, though originally benign, tend to undergo malignant degeneration. Finally, even those tumors which remain benign are not without serious hazard to the patient. They may become malignant in the clinical sense, for by pressure and displacement of neighboring organs and structures serious impairment of function or even death may result.

Not all mediastinal tumors are amenable to surgery. The lymphoblastoma group of lesions are the most commonly encountered growths not usually susceptible to surgical removal. Nevertheless, even in these and other nonsurgical neoplasms, a precise pathologic diagnosis is most important for reasons of prognosis and as a guide to intelligent irradiation therapy. It follows, therefore, that a great majority of mediastinal tumors demand open thoracotomy for removal of the growth, if possible, or biopsy if resection cannot be accomplished. Although the literature in recent years is replete with articles dealing with mediastinal tumors in general and with numerous specific types of lesions encountered in that region, little mention has been made of tuberculomas.

Burnham (1) credits Piazzi-Martini (2) with the original description of tuberculomas. Grace (3) achieved priority in the American literature, and he appears to have been the first to excise a tuberculoma. Blades and Dugan (4) removed a lesion of this type from the posterior mediastinum. Acid-fast organisms were found in the specimen. Two tuberculomas were removed by Paulson (5). In his report of mediastinal tumors removed at various Army thoracic surgical centers, Blades (6) mentions two tuberculomas. Sampson and his associates (7) have expressed the opinion that one of these cases was Blades' own; the other, one of Paulson's. It appears likely that their surmise is correct. Sampson and associates briefly reviewed the four previously reported cases and added four of their own. In all of the eight cases the lesions lay in the superior mediastinum. Five were on the right, three on the left. The tuberculin reaction was positive in three, not done in three, negative in one, and negative preoperatively and positive postoperatively in one. In the discussion of Sampson's paper, Woodruff (8) mentioned

a large cystic posterior mediastinal lesion which appeared to arise in the region of the bifurcation of the trachea and projected both to the right and left of the spine. Studies of the specimen were not complete and a histologic diagnosis was not given. On the same occasion Goldman (9) referred to a patient whose lesion showed some similarity to the lesion of one of Sampson's cases, but sufficient details were not given to warrant including it in the group of tuberculomas. Sampson and his co-workers (7) state that "The paucity of . . . reports leaves the questions of accurate preoperative diagnosis and of treatment (of 'tuberculomas') largely unanswered."

It should be emphasized that in the present report the lesions which have been described and accepted as mediastinal tuberculomas appear as *distinct tumors*. Neither roentgenographically nor surgically do they closely resemble ordinary tuberculous mediastinal nodes. The diagnostic measures usually undertaken in the investigation of any mediastinal lesion have been employed in an attempt to make an accurate preoperative determination of the nature of these masses. Few significant findings have been observed at *bronchoscopy*. *Barium studies* of the esophagus have been uniformly negative. *Angiocardiography* has been done almost routinely. In 4 cases, only slight lateral compression of the lower portion of the superior vena cava has been demonstrated. In one case (Case 5) complete obstruction of the right subclavian vein at its junction with the right innominate was observed. The collateral venous circulation was beautifully demonstrated. Angiocardiography in the remaining case (Case 2) failed to suggest the nature of the mass but did serve to show that it was probably extracardiac in origin. The value of contrast visualization of the heart and great vessels in the investigation of mediastinal masses has been rather well established by the papers of Sussman (10), Steinberg, Dotter, and Andrus (11), Steinberg and Dotter (12), and Dotter and Steinberg (13). The writers' experience with the procedure has led them to believe that it possesses real usefulness in establishing the exact location of mediastinal tuberculomas. Precise localization, as will be pointed out below, serves to indicate the probable nature of these "tumors." A *tracheogram* was done on only one case. Lipiodol was used as the contrast medium, and the close relationship of the mass to the trachea was verified. The procedure did not appear to add sufficient information to warrant its continued employment.

Roentgenographic studies consisted of both roentgenography and fluoroscopy. Posterior-anterior stereoscopic films employing both the standard and Potter-Bucky techniques were obtained in all cases and a right or left lateral film, depending on the side of the mediastinum to which the mass projected, was a part of the routine roentgenographic study.

The chest roentgenograms were of the greatest value in the diagnosis of these masses. In the frontal projection in the typical case a smoothly outlined, rounded area of increased density was seen projecting to the *right* of the mediastinum with its inferior border at the level of the crotch formed by the junction of the azygos vein with the superior vena cava. Stereoscopically, it could be seen that the lesion lay slightly anterior to the mid-plane of the chest as measured in the anterior-posterior diameter. The lateral view confirmed the position of the mass, showing

it to be situated along the anterolateral tracheal wall with the inferior margin at the level of the terminal portion of the azygos vein. Since the lesions characteristically appear quite round in this view also, their spherical or ovoid shape is established. The lesions are invariably solitary, in contradistinction to tuberculous mediastinal nodes which are usually multiple and commonly bilateral.

All except one of the mediastinal tuberculomas which were encountered have been of regular contour and sharply marginated. In this respect their roentgenographic appearance is quite unlike tuberculous adenitis and they simulate various true tumors of the mediastinum much more closely. Fluoroscopy corroborated the film evidence and showed that the lesions did not move with the lung on respiration and were therefore extrapulmonary in location.

Although some of the tumors in the present series appeared quite dense, in none of them could the presence of calcium be definitely shown on either the standard or Potter-Bucky films. In this connection, tomography was of some aid. In Case 6, the only instance in this series in which the tumor was not excised in whole or in part, associated findings were present which strongly suggested a tuberculous cause for the right mid-mediastinal mass. Although it could not be detected in the routine films, the presence of calcium was clearly demonstrated in the tomograms. This bit of evidence was considered helpful in determining the disposition of the case in question, for it is widely accepted that the finding of calcium is more characteristic of the granulomas, especially those of specific tuberculous etiology, than of other masses encountered in the mediastinum. Its value is lessened somewhat in the individual case by the equally well-known fact that other tumors in this location, including the teratoid lesions, aberrant goiters, neurogenic tumors, Hodgkin's disease (particularly the sclerosing type), aneurysms of the great vessels, thymic neoplasms, and others also at times show calcium deposits. Tomography is also important in differentiating solid from cavernous lesions.

A thorough investigation for the presence of active tuberculosis is of course mandatory when a lesion considered a possible or probable mediastinal tuberculoma is discovered. Haines (14) has emphasized that the recognition of tuberculous adenitis is ordinarily not difficult for "we are dealing with the local expression of a disease which has almost innumerable general symptoms, a sufficient number of which are usually present preceding the central chest lesion to readily distinguish them from other conditions found in the mediastinum." The experience of the present writers as well as that reported by others indicates that the foregoing viewpoint is not fully applicable to mediastinal tuberculomas as distinguished from ordinary tuberculous lymphadenitis of nodes in the mediastinum. It is obviously true, however, that the presence of proved tuberculosis elsewhere in the body, particularly in the lung, would cause serious consideration to be given to the possibility of a mediastinal mass being also on a tuberculous basis. A carefully taken history may disclose a previous tuberculous infection in the patient, a member of his immediate family, or a person with whom he has been closely associated. A thorough systemic investigation may reveal stigmata of healed tuberculosis.

Certain factors of a nonspecific nature carry some weight in the investigation of these masses. Because of the known high prevalence of tuberculosis in Negroes, American Indians, Filipinos, and Puerto Ricans, the possible tuberculous etiology of a mass of the type described when present in a person from one of these groups should receive serious consideration. A history of an antecedent "idiopathic" pleural effusion would be significant. Previous unexplained febrile episodes, loss of weight, or related general symptoms should be carefully evaluated. The age of the patients would carry some diagnostic weight. Four of the patients in the present series were in their early twenties and none was more than thirty years of age.

In the present series of cases, various skin tests have been employed routinely. These included the tuberculin, coccidiodin, and histoplasmin tests. In 4 of the patients, the tuberculin reaction was positive and in 2 patients it was negative. The tests for coccidioidomycosis and histoplasmosis were uniformly negative. A positive skin reaction to tuberculin offers helpful, but by no means conclusive, evidence of the nature of the lesion (7, 15, 16). On the other hand, a negative tuberculin reaction would mitigate strongly against the mass's being representative of a tuberculous granuloma though it would not definitely eliminate that possibility (16). The hemagglutination reaction, described by Middlebrook and Dubos in 1948, was not used in the investigation of these patients. The specificity of this reaction is still being studied. While it appears to offer considerable promise in the diagnosis of patients with clinically active, progressive tuberculosis, most authorities (17–20) agree that it is usually negative in patients who are free from spreading disease. Its value in the differential diagnosis of patients with tuberculomas would therefore be negligible.

To summarize the problem of diagnosis, it must be concluded that an accurate diagnosis of these lesions can only be established after thoracotomy with removal or biopsy of the mass. Routine histologic study, supplemented by special staining procedures and specific cultural methods applied to the excised specimen, will then usually establish the true nature of the lesion. On the other hand, attention is particularly invited to the fact that in five of the eight previously reported cases and in five of the six included in the present report, or a total of ten of fourteen cases, the masses occupied a very constant location in the mid-plane of the chest immediately above the level of the transverse portion of the azygos vein. In these ten cases, the mass lay in close proximity to the anterolateral tracheal wall and the lower portion of the superior vena cava (figure 25). Furthermore, most of these lesions were spherical or oval in shape, smooth in contour, and homogeneous in density. Even as to size, a number of these masses showed striking similarity. The cases illustrated by Sampson and co-workers (7) appeared much like the ones in the present report. The findings described have led us to conclude that, given a mass of the size, shape, position, and roentgenographic characteristics described, a mediastinal tuberculoma rates first consideration in the differential diagnosis. A history of tuberculosis, the presence of other evidence of that disease, and/or a positive tuberculin skin reaction would strengthen the possibility of the lesion's being a tuberculous granuloma.

Application of the term "tuberculoma" to these mediastinal granulomas has excited some critical comment. Although the writers are heartily in accord with the principle that correct descriptive nomenclature should be used wherever possible in denoting pathologic processes, they are unimpressed with the possible fallaciousness of using that designation for these masses. As a matter of fact, in so far as it has been possible to determine, there is no definition of the term *tuberculoma* which has proved acceptable to all, even when applied to lesions of the pulmonary parenchyma. Certainly the lesions which the writers have removed or biopsied were chronic granulomas and the same is true of similar cases reported by others. The finding of acid-fast bacilli by Ziehl-Neelsen stains of smears from

FIG. 25. Diagrammatic sketch showing the typical position of these lesions

the caseous centers of these lesions has in some instances, including two in the present series, established the tuberculous nature of the process. Perhaps these masses represent huge, solitary, circumscribed tuberculous lymph nodes in which all normal architecture has been destroyed. Possibly they represent true chronic encapsulated tuberculous abscesses arising from a focus in the mediastinal lymphatics, such as a small disrupted node. Regardless of their pathogenesis, the masses which the writers have studied are indisputable examples of chronic granulomas, and careful histologic study has shown no important difference between the microscopic appearance of these lesions and those known to be due to *M. tuberculosis*. Concerning the objection to calling them tuberculomas, what does it matter? The term tuberculoma in itself is indefinite enough, and even

pulmonary lesions to which that diagnosis is frequently applied with confidence often cannot be proved by bacteriologic methods to be due to *M. tuberculosis*. Hence, it appears that there is ample justification for calling these masses *tuberculomas* until their nature is more clearly defined or until a more suitable term is offered.

After detailed study of the tissue submitted from the 5 patients reported in this paper who were explored, the pathologist in this hospital made the following comments:

"The tuberculomas in Case 2 and Case 5 can be seen to be arising from chronic granulomatous lymphadenitis. In the others, however, no normal architecture remains to permit such an interpretation although that is the most probable origin. The diagnosis of tuberculosis was confirmed by Ziehl-Neelsen stains in Case 1 and Case 3. In Case 4 there was little or no remaining active inflammation, making it unlikely that tubercle bacilli could be demonstrated by routine methods. While the remaining two cases, Case 2 and Case 5, showed active chronic granulomatous processes with tubercle formation, no acid-fast bacilli were found. Although other pathogens, such as some of the deep fungi, or bacterial agents, for example *Pasteurella tularensis*, can produce similar histological changes, none of these organisms were recovered. Therefore, *Mycobacterium tuberculosis* was the most likely etiological agent."

The question has been raised of whether these lesions should be excised when discovered at exploration. It is definitely believed that they should be in spite of the fact that only one of the 6 patients reported herein had symptoms referrable to the mass. The writers have accepted the surgical philosophy that all patients with undiagnosed mediastinal tumors should be subjected to early thoracotomy for the purpose of both diagnosis and treatment, and do not question the necessity of exploring these patients. At operation a decision must be made concerning whether the mass should be biopsied or complete removal attempted. In view of the probable, if not certain, tuberculous etiology of these granulomatous lesions, it appears only reasonable that removal *in toto* would render far less likely the possibility of the development of an active tuberculous infection postoperatively. Regardless of the causative agent, it will doubtless prove more satisfying to the surgeon and reassuring to the patient to have the tumor completely enucleated than to leave a major portion of it resident in the chest. Even though it may be causing no symptoms at the time the diagnosis is made, there is no certain assurance that this circumstance will continue to prevail. In view of the fact that tuberculous lesions and other granulomas heal by fibrosis with scar tissue formation, and frequently with calcification, there is a distinct possibility that the effects of the healing process in the unoperated case may be equally as adverse as those caused by the active lesion (21). The report of Buckles (22) concerning the very serious disease that may be produced in the lung parenchyma as the result of bronchial obstruction due to large calcified hilar nodes has emphasized a little discussed but frequently encountered condition. Head and Moen (23) have also pointed out the late nontuberculous complications of calcified hilar nodes. Gray and Skinner (24) and Pilcher and Overholt (25) have described healing tuberculous processes as one of the common causes

of superior mediastinal obstruction and have emphasized the strangling effects which such lesions may produce.

Exceptions to the above principles will occasionally be necessary. In our experience, as well as in those recorded by others (Grace (3), Blades and Dugan (4), Sampson and associates (7), Paulson (5)), removal of these masses is not always a technically easy matter. In every case in the present series the mass was found to be more or less densely adherent to the important structures adjacent to it. The intense chronic inflammatory reaction excited by the granulomas is well known and it constituted an outstanding feature of the cases which we have explored. Conglomerate, matted-together masses have not been encountered at operation. As demonstrated by the roentgenographic studies, discrete solitary "tumors" have been found. The lesions appear to be definitely encapsulated but the capsule does not strip with ease. In no case have the writers seen noteworthy associated lymphadenopathy. Although they have been fortunate enough to avoid tearing adjacent vessels and the sacrifice of important nerves, the tumors have been firmly adherent to vascular organs, nerve tissues, and segments of the tracheobronchial tree. Removal of the mass intact has required a prolonged, tedious, and rather difficult dissection. In one case (Case 5) it appeared that complete removal would constitute an almost impossible surgical feat; certainly it could have been undertaken only with the greatest hazard of serious injury to important mediastinal structures. In that case it was decided to remove only the easily accessible portions of the mass after frozen section examination of a biopsy specimen had shown it to be a benign granuloma. It was gratifying and frankly surprising to find on postoperative contrast visualization studies that the complete obstruction of the right subclavian vein had been relieved. The part that the prolonged postoperative course of streptomycin and para-aminosalicylic acid may have played in accomplishing this result is not minimized.

It is believed that the possible tragedy of an occasional erroneous diagnosis would so far outweigh the minimal risk of routine thoracic exploration in lesions thought likely to be mediastinal granulomas that operation should be advised in all such cases unless there is the very strongest possible presumptive evidence that the mass represents a quiescent or healed tuberculous focus. Enumeration of the details of the case is not pertinent to this paper but figures 26 and 27 illustrate a lesion which was clinically indistinguishable from the tuberculomas of the present series *except* that it lay on the *left* and was situated slightly more anteriorly than a typical tuberculoma. This mediastinal mass was excised from a 24-year-old white male and on histologic examination proved to be a highly malignant thymic carcinoma. Until it becomes possible to make a positive diagnosis of these lesions without surgery, continued observation as a method of management will rarely be justifiable.

No authentic statement can be made as to the proper postoperative management of these patients. In the present series the lesions were considered to be of tuberculous origin and, motivated by the desire to err on the side of safety, the patients were maintained on strict bed rest for three months postoperatively. Thereafter they were all allowed out of bed for progressively increasing periods

until they had completed six months of postoperative medical care before return to full activity. Streptomycin, 1 gm. daily, and PAS, 12 gm. daily, were given postoperatively in courses which have varied in duration from six weeks to four months. All patients reported in this paper were personnel on active duty in the Armed Forces. Five of the 6 patients have been returned to full duty, and it is anticipated that the remaining person will receive similar disposition upon completion of his arbitrarily chosen six-month period of postoperative rest. There is no assurance that the regimen which has been adopted is either necessary or advisable. Clarification of this point must await the report, with long-term follow-up, of similar cases treated by various methods.

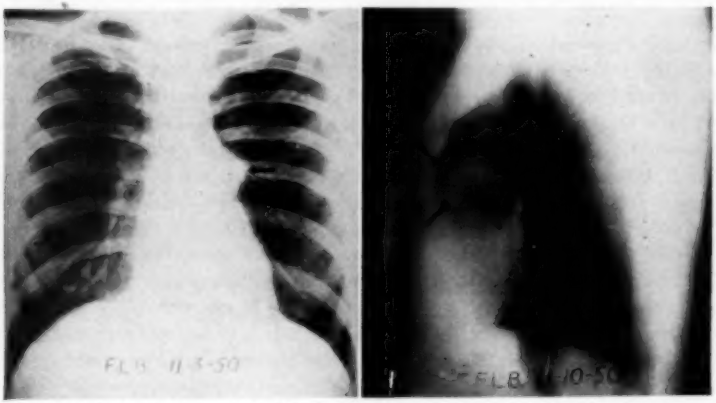


FIG. 26. (Left) Posterior-anterior chest roentgenogram showing a mass closely simulating in its roentgenographic appearance a mediastinal tuberculoma except that it projects from the *left* hilum. This proved to be a thymic carcinoma.

FIG. 27 (Right) Lateral tomogram at the 10-centimeter level showing the mass illustrated in figure 26 to lie somewhat anterior to the mid-plane of the chest.

SUMMARY

Six cases of mediastinal tuberculosis have been reported in detail and the eight previously reported cases and two additional possible cases of this entity have been reviewed.

The presence of an oval or spherical, smoothly outlined, and sharply circumscribed mass of homogeneous density or containing calcium deposits, projecting to the right from the mediastinum with its inferior border at the level of the transverse portion of the azygos vein and situated near the mid-plane of the chest, strongly suggests the possibility that the lesion represents a mediastinal tuberculoma.

As there is no method whereby a positive diagnosis of mediastinal tuberculoma can be made preoperatively, it is believed that virtually all of these patients should be subjected to exploratory thoracotomy. Removal of the mass at the time of operation is advocated if it can be accomplished with reasonable safety.

The characteristic histologic picture of these lesions is briefly described and illustrated. In the present state of our knowledge concerning masses of this type, the name tuberculoma appears to be an acceptable descriptive term for clinical purposes.

SUMARIO

Tuberculomas del Mediastino

En este repaso del tema de los tuberculomas mediastínicos, menciónanse los 8 casos previamente comunicados y otros 2 posibles casos y pormenorízanse 6 casos nuevos.

Indícase que, hasta demostrar lo contrario, se considere como tuberculoma del mediastino toda tumefacción oval o esférica de contornos lisos y netamente circunscrita de espesor homogéneo o que contenga depósitos de calcio, que proyecte hacia la derecha del mediastino, tenga su borde inferior a la altura de la porción horizontal de la vena acigos y esté situada cerca del plano medio del tórax.

Por no haber método alguno que permita hacer un diagnóstico positivo preoperatoriamente, parece que virtualmente todos estos enfermos deben ser objeto de una toracotomía exploradora. Recomiéndase la extirpación de la tumefacción al hacer dicha operación si puede ejecutarse sin mayor temor.

Descríbese sucintamente y reprodúcese el típico cuadro histológico de estas lesiones. En el actual estado de nuestros conocimientos acerca de esta clase de tumefacciones, el nombre de tuberculoma parece constituir un aceptable término descriptivo para fines clínicos.

RESUME

Tuberculose du médiastin

Discussion de la tuberculose médiastinale. Les huits cas précedemment rapportés et deux cas additionnels possibles ont été mentionnés; six cas nouveaux ont été décrits en détail.

Il est proposé que soit considéré comme tuberculome médiastinal, jusqu' à preuve du contraire, toute masse de forme ovale ou sphérique à bords réguliers, nettement délimitée et de densité homogène ou renfermant des dépots de calcium, située au milieu du thorax, débordant le médiastin vers la droite et dont le bord inférieur est au niveau de la veine azygos.

Comme il n'existe aucune méthode permettant un diagnostic préoperatoire, les auteurs pensent que pratiquement tout ces malades doivent être soumis à la thoracothomie exploratrice. L'exérèse de la masse au moment de l'intervention est conseillée si celle-ci peut-être effectuée avec une certaine sécurité.

L'aspect histologique caractéristique de ces lésions est brièvement décrit et illustré. En l'état actuel de nos connaissances sur ces masses le terme descriptif de tuberculome paraît acceptable pour les désigner cliniquement.

Acknowledgment

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THE ENUMERATION OF VIABLE TUBERCLE BACILLI BY SURFACE PLATE COUNTS[1]

FRANK FENNER

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INTRODUCTION

In the investigation of many of the phenomena associated with the multiplication of infectious agents, either in the test tube or in the experimental animal, it is advisable to express the infective dose and the degree of multiplication which may occur in the culture medium or in different organs of infected animals in terms of the numbers of viable bacteria or infectious particles concerned. In the study of tuberculosis, however, a subject on which the volume of the experimental work probably exceeds that devoted to any other infectious disease, it is still common practice to express dosage as the weight of the bacillary mass inoculated, irrespective of both the physiologic state of the bacteria used and the number of viable bacterial cells in the inoculum. This stems directly from the lack of satisfactory methods for dispersing cultures and for counting viable tubercle bacilli.

The introduction by Dubos (1) of the wetting agent Tween 80[2] has overcome the first difficulty, that of obtaining a well-dispersed suspension of bacteria. It is true that Tween-albumin cultures do not consist entirely of isolated bacterial cells but, if they are lightly centrifuged or filtered through paper, the supernate or filtrate will contain few clumps of more than three or four bacilli. It is probable that, like isolated bacterial cells, small clumps behave as single infectious units on introduction into the animal body. Bloch (2), for example, failed to demonstrate any difference in the virulence of well-dispersed or "clumpy" suspensions of tubercle bacilli inoculated intravenously in mice when each suspension contained the same number of bacterial cells in the same physiologic state, although striking differences occurred when similarly dispersed cultures which had been incubated for different periods were used. The results of a study of the state of dispersion of unfiltered Tween-albumin cultures of several strains of mammalian tubercle bacilli (table 3) showed that in most of them at least 80 per cent of the bacterial units or groups contained less than five microorganisms.

None of the methods commonly employed for counting viable tubercle bacilli gives consistently satisfactory results. Wells and Lurie and their colleagues have carried out many counts of viable organisms during their investigations of airborne and other infections in rabbits, but their methods do not lend them-

[1] From The Department of Microbiology, The John Curtin School of Medical Research, The Australian National University, Canberra, Australia.

[2] Tween 80 is the proprietary name of the polyoxythylene derivative of Sorbitan monooleate, obtainable from the Hill Top Laboratories, Inc., 921 Wm. H. Taft Road, Cincinnati 6, Ohio.

selves to adequate statistical manipulation; the time required for obtaining results is unduly long; and, as Rich (3) has pointed out, the variability of the results obtained in parallel experiments on different animals was great. Apart from two brief notes (4, 5) nothing has been published by these authors concerning the assessment of the reliability of the cultural methods employed.

A general discussion of the problems involved in the enumeration of viable tubercle bacilli, both from cultures and from the tissues of infected animals, has been presented elsewhere (6). A brief description was given in that study of the results obtained when surface plate counts of tubercle bacilli were made on the oleic acid-albumin-agar medium of Dubos. Most of the experiments then described were carried out with well-dispersed Tween-albumin cultures of standard laboratory strains of tubercle bacilli which contained many small clumps as well as isolated bacterial cells. Two criticisms might be advanced concerning the general validity of these experiments. First, it is conceivable that the viable counts obtained recorded only the numbers of clumps containing two, three, or more bacilli, rather than the total number of bacterial units, whether isolated bacterial cells or small clumps. Second, the strains used had been cultivated for very long periods on laboratory media, including regular passage in the Tween-albumin liquid medium for three years. The excellent results obtained with these strains might not, therefore, be applicable to strains of tubercle bacilli freshly isolated from animal tissues.

Experiments were therefore carried out with filtered suspensions of a number of strains of mammalian tubercle bacilli, including some just isolated from infected animals or men. In the present report a detailed description is given of the technique used for the enumeration of viable tubercle bacilli of these different strains by surface plate counts on oleic acid-albumin-agar. The experiments were carried out with filtrates which contained predominantly single bacterial cells, as well as with the untreated cultures from which the filtrates were obtained. The results which are recorded show that, even with such well-dispersed suspensions, viable mammalian tubercle bacilli of a number of different strains can be counted by the surface drop method with much the same degree of accuracy and ease as many other bacteria (7). Moreover, comparisons of the viable counts with total counts made in a Petroff-Hausser chamber indicate that with most strains isolated bacterial cells regularly produced colonies on the surface of this solid medium.

MATERIALS

Bacterial strains: The following bacterial strains were studied:

(a) H37Rv and H37Ra strains of human-type tubercle bacilli, which had been maintained for four years by serial transfer in Tween-bovine-albumin liquid medium.[1]

(b) "Amesaga" strain of human-type tubercle bacilli, which had been maintained for twelve months by serial transfer in Tween-albumin medium.[1]

(c) "Pratt" strain of human-type tubercle bacilli, isolated from human sputum on the Löwenstein medium by Dr. Hilda Gardner of the Royal Melbourne Hospital. The first subculture of this strain in Tween-albumin medium was used for the experiments described.

[1] Provided by Dr. R. J. Dubos, Rockefeller Institute for Medical Research, New York, New York.

(d) "Ravenel" strain of bovine-type tubercle bacilli.[3] This had been maintained for four years by serial transfer in Tween-albumin medium.

(e) "Bovine 1" strain of bovine tubercle bacilli, isolated on the Löwenstein medium from the infected lymph node of a slaughtered cow.[4] The first subculture in Tween-albumin medium was used for the experiments described.

(f) BCG, strain F46 of the State Serum Institute, Copenhagen.[5] This had been maintained for six months by serial transfer in Tween-albumin medium.

(g) *Mycobacterium muris*, strain OV164.[6] This had been maintained for six months by serial transfer in Tween-albumin medium.

(h) *Mycobacterium ulcerans*[7], strain "RT" (8). This strain had been maintained for eighteen months by serial transfer in Tween-albumin medium.

Culture media: Two varieties of semi-synthetic culture medium containing bovine albumin fraction V were used:

(a) Oleic acid-bovine albumin-agar medium, containing no Tween 80, was poured in Petri dishes and used for the performance of the surface drop counts. The formula of the medium and its preparation have been described several times (6, 9).

(b) Tween 80-bovine albumin liquid medium (9) was used in 5 ml. volumes in one-ounce screw-topped bottles ("universal containers") both for the preparation of stock cultures and for viable counts by the dilution method in liquid media. Stock cultures were sometimes grown in media lacking glucose, following Dubos' demonstration (10) of the fall in the viable count of tubercle bacilli after continued incubation in media containing glucose, but in the experiment upon dilution counts 0.5 per cent glucose was included in the medium.

Comparisons were also made with Lurie's modification of Löwenstein's medium (11), the details of the preparation of which were kindly supplied by Dr. Max B. Lurie of the Henry Phipps Institute, Philadelphia. This medium was dispensed in rubber-stoppered test tubes.

METHODS

Preparation of bacterial suspensions: Except in the experiments upon the reliability of the dilution method (Experiment 1) and on the choice of diluent (Experiment 2), in which filtered preparations only of strain H37Rv were used, parallel counts were made with the unfiltered culture and with the filtrate obtained after this had been filtered twice through Whatman Number 42 filter papers.

Total bacterial counts: Counts were made in a Petroff-Hausser chamber of the total number of bacterial units in the suspensions, using a Zeiss "Lumipan" microscope with 1.5× magnifying draw-tube prisms, achromatic 0.40 20× objective, 20× compensating oculars, and dark phase contrast illumination. Each single bacterial cell and each group of bacteria were counted as one bacterial unit. Virulent tubercle bacilli were first killed by the addition of an equal volume of a 10 per cent aqueous solution of formalin. This did not alter the state of dispersion of the bacteria.

Counts of acid-fast bacilli and their state of dispersion: Crandall (12) described a method of counting the number of tubercle bacilli in dilute suspensions, containing as few as 300,000 organisms, which depended on counting all of the stained bacilli present in a carefully measured very small drop of the suspension. A modification of his technique was devised, using the "Agla" micrometer syringe[8] (13) to which a length of bent glass tubing drawn to a fine point was attached. Drops of 0.0004 ml. volume of a dilution estimated to give approximately 100 bacterial units per drop were placed on a slide previously coated with a film of 50 per cent serum. The prepared slide, which rested upon the draw-tube of a microscope stand, was raised to the fine glass point and the micrometer adjusted. Twelve drops were

[3] Provided by Mr. A. G. Heywood of the Victorian Department of Agriculture.

[4] Supplied by Dr. E. A. North of the Commonwealth Serum Laboratories, Melbourne, Australia.

[5] Supplied by the Commonwealth Serum Laboratories, Melbourne, Australia.

[7] This is the name which has been proposed for the newly discovered *Mycobacterium* by MacCallum, Tolhurst, and Buckle (personal communication).

usually placed on each slide, and these were then stained by the Ziehl-Neelsen method. Inspection with the low power showed that with care none of the film was detached or washed off during the staining process. In several of these drops the total number of units (single organisms or small clumps) of acid-fast bacteria were counted, using the oil-immersion objective (achromatic 1.25 HI 90×) and 10× Huyghenian oculars, and a record was kept of the number of bacterial cells seen as isolated bacilli and in groups of 2, 3, 4, and 5 or more cells.

Inoculation of the plates: For surface plate counts the surface of the solid medium must be free of excess moisture, and this was ensured by pouring the plates with the agar as cool as possible and then drying them for twenty-four hours in an incubator at 37°C. The drying time may be considerably prolonged without interfering with the sensitivity of the medium.

In preliminary experiments, several methods of inoculating the plates were compared (6). The counts obtained when 0.1 ml. was inoculated from a one-milliliter pipette on the surface of a two-inch plate did not differ significantly from the counts obtained when 0.02 ml. inoculum was dropped on the surface of plates either with a 0.25-milliliter syringe and 20-gauge needle with a dropping pipette or with a serologic pipette graduated in thousandths of a milliliter. Snyder (14) showed that in experiments on viable counts with dysentery bacilli the accuracy of measurement of the inoculum provided a negligible contribution to the total error of the count, and the method eventually adopted in these experiments was based upon Miles and Misra's (15) surface drop count and consisted of placing one drop of approximately 0.02 ml., measured from a dropping pipette constructed according to Donald's (16) directions, on each of eight sections of a 3½- or 4½-inch plate, with two more drops in the center. In this way ten replicates were obtained on one plate. Each drop usually spread to cover a circular area with a diameter of approximately 15 mm. The surface was allowed to dry, with the plate on a horizontal shelf in the incubator, and the plates were then sealed with adhesive strapping. The exact volume of the drops used in each titration was determined by counting the number of drops in an accurately measured 0.5 ml. of one of the dilutions of culture used in the titration.

Counting the colonies: Incubation at 37°C. (or 33°C. in the case of *Mycobacterium ulcerans*) proceeded for various periods before counts were made. Readings were made with a binocular stereoscopic microscope at a magnification of 10×, using incident illumination. A higher power objective was used in the case of doubtful or very small colonies. Counts were repeated a week after colonies appeared to be well grown. If any new colonies had appeared at this second count, incubation was continued for a further week and another count then made, and this process was repeated until steady or falling counts were obtained. Confluence of the larger colonies often led to a reduction in the later counts, and the optimum time for reading mentioned in table 9 was the occasion upon which no new colonies subsequently appeared and the existing colonies were still discrete and easily distinguishable.

Following Lurie's recommendations (11), counts were made of the colonies on the Lurie-Löwenstein medium at weekly intervals until no increase in the number of colonies was noted on two successive readings.

[8] Supplied by Messrs. Burroughs Wellcome & Co., London, England.

Statistical treatment of data: The following statistics were calculated from the results of the colony counts, where x = number of colonies per drop area, n = number of replicate drops.

(1) mean, $\bar{x} = \frac{\Sigma x}{n}$

(2) variance, $s^2 = \frac{\Sigma(x - \bar{x})^2}{n - 1}$

(3) standard error of the mean, $s_{\bar{x}} = \frac{s}{\sqrt{n}}$

(4) coefficient of variation of the mean, $C_{\bar{x}} = \frac{100 s_{\bar{x}}}{\bar{x}}$

Jennison and Wadsworth (17) have pointed out that the total error of bacterial plate counts consists of two components: the dilution error, due to variations in the volumes delivered from one-milliliter and ten-milliliter pipettes when making up the dilutions; and the distribution error, which is the standard error of the mean (3) calculated from the colony counts. Expressed as percentage standard error, or coefficient of variation of the mean, the total error can be calculated from the formula

$$\text{percentage total error} = \sqrt{(\text{percentage dilution error})^2 + (\text{percentage distribution error})^2}.$$

The percentage dilution error in the present experiments was determined but, in the various tables recording the results of viable counts, only the distribution error (standard error of the mean) is recorded. However, the total error was calculated when determining the significance of differences in mean counts derived from different dilution operations, and the ratio of counts obtained by one method with those obtained by another, which involved a different series of dilution steps.

To compare the effects of different treatments (dilution operations, diluent solutions, et cetera) upon the same bacterial suspension, an analysis of variance was carried out on the colony counts obtained. When the mean counts were less than 50 the data were transformed, using the square root transformation, in order to make the variance independent of the mean (18). Before proceeding to pool any variances that showed apparent discrepancies, Bartlett's test of homogeneity (18) was applied.

If the variance ratio, $F = \frac{s_1^2}{s^2}$ (where s_1^2 is the "between samples" estimate of variance and s^2 the pooled "within samples" estimate of variance), was significant, the appropriate pairs of means (or transformed means) were compared by referring the value of

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

to the t tables; $\bar{x}_1$ and $\bar{x}_2$ being the mean counts to be compared, and n_1 and n_2 the corresponding numbers of replicates. The number of degrees of freedom is equal to the sum of the degrees of freedom of each set of replicate counts used for the determination of the "within samples" estimate of variance, s^2.

In order to determine the significance of differences obtained by plating different dilutions of the same suspension, the means and standard deviations were adjusted to allow for the tenfold difference of dilution by dividing each by 10, and the "t" test employed on these adjusted figures.

Since the classical investigation of Fisher, Thornton, and Mackenzie (19), it has been known that the distribution of bacteria in replicate aliquots of a bacterial suspension appears to follow the Poisson distribution (34) and tests of conformity of the results of counts to this distribution have been used to determine the suitability of nutrient media for different bacteria (15, 20). Experiments with strain H37Rv designed to test the correspondence of the viable counts with the Poisson distribution are recorded later in this paper. They showed that the distribution of colonies from replicate drops of a dilute suspension of bacilli conformed closely to the Poisson distribution.

TABLE 1

*Smallest Differences Between Comparable Mean Counts Which Will Be Significant at the 5 Per Cent Level for Varying Combinations of n (the Number of Replicates) and $\bar{x}$ (Mean Count)**

n	$\bar{x}$	SMALLEST PERCENTAGE DIFFERENCE SIGNIFICANT AT 5% LEVEL $\left(=\frac{1.96 \times 100}{\sqrt{(n)\bar{x}}}\right)$
10	10	20
	20	14
	30	11
	40	10
	50	9
20	10	14
	20	10
	30	8
	40	7
	50	6
30	10	11
	20	8
	30	7
	40	6
	50	5

* This refers particularly to surface-drop counts of viable tubercle bacilli but similar reasoning also applies to total counts made in a counting chamber.

It may be valuable to consider one consequence of the Poisson distribution of bacteria in replicate aliquots of a suspension which is important in counting experiments similar to those described in the present report. The Poisson distribution is defined by a single parameter, the mean m (the standard deviation being equal to $\sqrt{m}$). Assuming that m is sufficiently large for normal theory to be applicable, it is possible to investigate the smallest possible differences in the mean count which would be judged significant (at, for example, the 5 per cent level) for any combination of n (number of replicates) and $\bar{x}$ (mean count). Table 1 sets out such figures for various combinations of n and $\bar{x}$ likely to be encountered in surface drop plate counts, expressed as percentage differences in comparable counts.

It is apparent that the sensitivity of the method is proportional to the square root of the total number of colonies counted. For a given number of replications, therefore, the greater the number of colonies per drop area, the greater will be the sensitivity. Against this must be set the limitation imposed by the small size of the drop area and the necessity to avoid overcrowding and confluence of colonies.

EXPERIMENTAL RESULTS

The accuracy of total counts made in the Petroff-Hausser chamber: To determine the reproducibility of bacterial counts made with the Petroff-Hausser chamber,

TABLE 2

Total Bacterial Counts Made in a Petroff-Hausser Chamber and in a Stained Drop of Known Small Volume

COUNTING METHOD	DILUTION OF PREPARATION	UNIT AREA COUNTED	MEAN COUNT PER UNIT AREA	"PERCENTAGE ERROR" (5 PER CENT LEVEL)		TOTAL COUNT	$\frac{s‡}{\sqrt{\bar{x}'}}$ MEAN OF 10 REPLICATES
				Actual $\frac{(200 s_{\bar{x}})†}{\bar{x}}$ (= 2 × coefficient of variation)	Theoretical $\left(\frac{196}{\sqrt{(n\bar{x})}}\right)$*		
				per cent	*per cent*		
Petroff-Hausser chamber	Undiluted	16 small squares (5 replicates)	41.8	7.7	4.3	1.05×10^9/ml.	0.82
Petroff-Hausser chamber	1:16 dilution	80 small squares (5 replicates)	15.0	7.4	7.2	1.20×10^7/ml.	1.11
Stained drop	1:50 dilution	0.0004 ml. volume	114.3	14.0	6.1	1.4×10^7/ml.	—

* See table 1.

† $\bar{x}_1$, $s_{\bar{x}}$, = means and standard errors of replicate totals.

‡ $\bar{x}_1$, s_1 = means and standard deviations of individual results of each replicate count.

ten successive counts of one preparation of BCG were made at each of two dilutions, using the same counting chamber and coverslip on each occasion. With the more concentrated suspension, all bacteria were counted in the 80 small squares (five large ones) distributed symmetrically over the total area of 400 sq. mm.; with the more dilute preparation, all bacteria were counted in the 400 small squares, totals being recorded for each horizontal row of five large squares. The results obtained are given in table 2.

The results did not differ greatly from the theoretical variability, especially with the more dilute preparation. Comparison of the means and standard deviations of each of the ten replicate counts showed that in each case the ratio, $\frac{s}{\sqrt{\bar{x}_1}}$, was not far from unity. The number of unit areas counted in each operation

(five) was too small to expect any closer conformity of this ratio to unity than was obtained.

In the principal experiments, total counts in the Petroff-Hausser chamber were made primarily with the object of deciding which tenfold dilutions should be used in the plate counts, and two replicate counts, each of five large squares or five rows of large squares in the case of the more dilute preparations, were made on each preparation. In table 10 the standard errors of these counts are recorded. The magnitude of the standard error varied considerably in different counts, and the average percentage standard error or coefficient of variation of the mean was 7.6 per cent, which is about twice the errors obtained when ten replicate counts were made of the same preparation.

The accuracy of total and differential counts of acid-fast bacteria in stained small drops: To test the reproducibility of the total and differential (state of dispersion) counts made on the stained drops of culture dilutions (after Crandall (12)), differential counts were carried out on nine replicate drops each of 0.0004 ml. of a 1 to 50 dilution of the unfiltered culture of BCG used above. The total count obtained was somewhat greater than that obtained with the counting chamber and the variability in the counts obtained from successive drops was also somewhat greater, the coefficient of variation being 7 per cent. The main purpose of examination of the stained drops was to determine the state of dispersion of the organisms. By reducing the numbers of groups of 1, 2, 3, 4, and 5 or more bacilli obtained in each count to percentages of the total count per drop, it was possible to compare the estimates of the dispersion made with each of the nine drops counted. The average standard error for the five categories was 1.26, giving an average coefficient of variation of the mean of 6.3 per cent.

In the stained drop counts included in table 10, differential counts were made on 2 to 5 drops, primarily to determine the state of dispersion of the bacilli (table 3). Variations in the magnitude of the standard errors were considerable, and the average coefficient of variation of the mean from the 15 specimens on which calculation was possible was 7.4 per cent, almost the same as that obtained with the chamber count.

Particulars of cultures used: In table 3 are presented the data relating to the presence or absence of glucose in the liquid culture medium, the age of the culture used, and its state of dispersion before and after filtration through filter paper.

Preparation of dilutions (Experiment 1): Serial tenfold dilutions of the bacterial suspensions were prepared according to the recommendations of Wilson (22), separate one-milliliter pipettes being used for each dilution. Transfers of 1.0 ml. were made into 9.0 blanks, after first pipetting the mixture up and down twenty times.

Estimates were made of the accuracy with which volumes of 1.0 ml. and 9.0 ml. were delivered from the one-milliliter and ten-milliliter delivery pipettes used for making the dilutions. Operating with the same speed and degree of care employed in the dilution operations of the experimental counts, the volume of distilled water discharged into weighing bottles was measured, 12 replicate

measurements being made with two representative samples of each type of pipette. The error involved in the measurements of the volumes from the pipette, expressed as percentage standard deviation, was approximately 2 per cent for

TABLE 3

State of Dispersion of Various Mycobacteria Grown in Tween-Albumin Liquid Medium Before and After Filtration Twice through Filter Paper

STRAIN		AGE OF CULTURE	GLUCOSE IN CULTURE MEDIUM	TREATMENT	NUMBER OF BACILLI IN BACTERIAL UNITS (PER CENT)				
					1	2	3	4	5-20
		days							
	H37Rv*	32	+	Filtered	75	15	6	2	2
	H37Rv†	33	+	Filtered	79	15	4	1	1
Human type	H37Rv	10	−	Unfiltered	32	21	20	12	15
				Filtered	58	24	7	7	4
	H37Ra	8	+	Unfiltered	15	14	11	10	50
				Filtered	56	32	8	2	2
	Amezaga	5	+	Unfiltered	46	20	7	9	18
				Filtered	89	9	0	1	1
	Pratt	14	+	Unfiltered	41	22	11	10	16
				Filtered	73	18	4	3	2
Bovine type	Ravenel	10	−	Unfiltered	31	16	14	9	30
				Filtered	40	25	13	9	13
	Bovine 1	16	−	Unfiltered	31	32	18	10	9
				Filtered	53	26	12	5	4
	BCG	10	+	Unfiltered	31	23	19	8	19
				Filtered	50	28	11	5	6
Murine type	OV164	14	+	Unfiltered	29	28	17	9	17
				Filtered	73	21	3	2	1
M. ulcerans	RT	32	+	Unfiltered	18	19	15	11	37
				Filtered	62	19	8	6	5

* Experiment 1 (dilution technique).
† Experiment 2 (choice of diluent).

both the one-milliliter and the ten-milliliter pipettes. Reference to Jennison and Wadsworth's (17) table indicates that maximum dilution errors (expressed as coefficients of variation) of 11.4 per cent and 14.2 per cent might be expected in the preparation of the 10^{-4} and 10^{-5} dilutions, due to errors in the graduation and use of the pipettes.

The results of an experiment (Experiment 1) on the reproducibility of the

dilution process are given in table 4. The bacterial suspension was a filtered culture of H37Rv, containing about 75 per cent of isolated bacterial cells, and the diluent used was 0.1 per cent bovine albumin in distilled water. Using the same bacterial suspension, the dilution process was repeated three times and counts were made with each preparation on oleic acid-albumin-agar, using the 10^{-3} and 10^{-4} dilutions. Portions of the 10^{-3} and 10^{-4} tubes of one preparation (A) were then each diluted to nine-tenths and two-thirds of the original concentra-

TABLE 4

Comparison of Results Obtained with a Filtered Culture of H37Rv on Replicate Dilution Operations in 0.1 Per Cent Bovine Albumin (Experiment 1)

PREPARATION	DILUTION				
	10^{-3}			10^{-4}	
	Number of replicates (n)	Mean count ($\bar{x}$)	Transformed mean count $\frac{\Sigma\sqrt{x}}{n}$	Replicates (n)	Mean count ($\bar{x}$)
A	30	8.0	2.80	30	78.3
B	25	7.6	2.73	29	72.2
C	30	7.7	2.76	37	79.9
D = $\frac{9}{10}$ A	34	5.8	2.37	30	48.8
E = $\frac{6}{10}$ A	30	7.1	2.63	42	60.1

TABLE 5

Analysis of Variance on Data of Table 4 (Experiment 1)

DILUTION	SOURCE	DEGREES OF FREEDOM	MEAN SQUARE	F
10^{-3} (Transformed data)	Between preparations	4	0.9555	5.89*
	Within preparations	144	0.1622	
10^{-4} (Original data)	Between preparations	4	4857.5	57.2*
	Within preparations	163	84.92	

*Highly significant ($P < 0.01$).

tion with 0.1 per cent bovine albumin, and counts were made. The plates were incubated for twenty-one days at 37°C. before reading.

The analyses were made, using the data from the count and ignoring the dilution error which could, as indicated earlier, be as great as 11.4 per cent for the 10^{-4} dilution and 14.2 per cent for the 10^{-3} dilution. The preparation of D and E from A did not, of course, involve a dilution error of this magnitude, as only one dilution step was made in each case instead of four or five. Within the preparations the individual variances were found to be compatible when tested by the Bartlett test, and an analysis of variance was therefore applied to the data. The results, set out in table 5, indicate that there are significant differences between certain of the colony counts at both dilutions.

The t test was applied to the differences between individual means (transformed data) at the 10^{-5} dilution, and showed significance only in comparisons involving preparation D. For example, the value of t for the comparison of the means of preparations A and D was found to be 4.30, which for 144 degrees of freedom is highly significant. No significant difference existed between the results of the three replicate dilution operations A, B, and C; nor was the 11 per cent difference between A and E significant (see table 1).

The colony count obtained at the 10^{-4} dilution of one of the replicate dilution operations, B, was distinctly lower than the other two and somewhat lower than might be expected from consideration of the counts at 10^{-5}. If, however, the percentage dilution error, 11.4 per cent, is included in the estimation of the total error and the standard errors derived from this used for estimating t, there is no significant difference between any of the replicate dilution operations A, B, and C.

With the larger mean counts obtained at the 10^{-4} dilution, the 12 per cent difference between A and D ($D = \frac{9}{10}A$) was highly significant, as, of course, was the 38 per cent difference between A and E ($E = \frac{3}{4}A$).

One test of the satisfactory nature of the dilution technique is the correspondence of the mean counts obtained with successive tenfold dilutions of the same preparation. Comparison by the t test of the adjusted means for the two dilutions given in table 4 showed that in each case there was satisfactory agreement.

This experiment gave very satisfactory results. Replicate dilution operations of the same preparation gave approximately the same count and, when the concentration of bacilli was reduced by 10 and 33 per cent, there were corresponding falls in the colony counts.

Choice of diluent (*Experiment* 2)*:* Drea (23) showed that *Mycobacteria* are very susceptible to the toxic effects of traces of fatty acids such as often contaminate laboratory glassware, and Dubos (24) has recently reported studies upon the effects of organic acids upon the growth of tubercle bacilli. Davis and Dubos (25) found that the effect of these toxic substances could be neutralized by bovine albumin. In table 6 are recorded the colony counts of a filtered suspension of H37Rv, containing 79 per cent of isolated bacterial cells, diluted out in distilled water, 0.1 per cent bovine albumin in distilled water, and M/15 disodium hydrogen phosphate, which has been used extensively by Lurie following Jennings' (26) demonstration that spontaneous agglutination of tubercle bacilli did not occur in this diluent. In order to eliminate any protective effect due to carry-over of albumin from the culture medium, which had apparently been operative in a previous experiment, the bacterial suspension used for this experiment was spun out of the culture medium and resuspended in fresh Dubos liquid medium containing Tween 80 but no albumin before being filtered through paper. Plates were inoculated immediately when the dilutions had been made up and again after they had remained for two and twenty-four hours, respectively, at room temperature (20°C.). Before each of the later inoculations the contents of the tubes were thoroughly mixed with a pipette.

If the initial viable counts of the three preparations (F-O, G-O, H-O) are con-

sidered first, it is apparent that they do not deviate greatly from one another at either the 10^{-4} or 10^{-5} dilution. Since these counts were obtained by different

TABLE 6

Comparison of the Colony Counts of a Filtered Suspension of H37Rv, Diluted in Three Different Diluents and Tested After Standing at Room Temperature (20°C.) for Various Intervals (Experiment 2). Counts Corrected to a Uniform Drop Size of 0.02 Ml.

DILUENT	INTERVAL	DILUTION							
		10^{-5}			10^{-4}			10^{-3}	
		Number of replicates	Mean counts	Standard errors	Number of replicates	Mean counts	Standard errors	Number of replicates	Mean counts
	hours								
0.1 per cent bovine albumin (F)	0	30	1.3	0.2372	30	20.7	0.8556		
	2	30	1.7	0.1850	20	19.1	0.9318		
	24	30	1.3	0.2151	26	14.1	0.7041		
Distilled water (G)	0	20	1.9	0.2542	30	24.2	0.8684		
	2	30	0	—	30	17.0	0.6460		
	24		—		30	0	—	30	0.03
Disodium hydrogen phosphate (H)	0	30	2.2	0.3058	26	19.8	0.6523		
	2	30	0.9	0.2604	30	13.8	0.6078		
	24		—		30	0	—	30	0.7

TABLE 7

The Significance of Differences Between Corrected Mean Counts of a Preparation of H37Rv Standing for Various Periods at Room Temperature after Dilution in Different Diluents (Experiment 2)

RESULTS COMPARED	DILUTION	DIFFERENCES BETWEEN MEANS	STANDARD ERRORS OF DIFFERENCES	"STUDENTS" RATIO (t)	SIGNIFICANCE
F-O*, G-O	10^{-5}	−0.6	0.4771†	1.2575	N. S.‡
	10^{-4}	−3.5	3.8301†	0.9208	N. S.
F-O, H-O	10^{-5}	−0.9	0.5291†	1.7010	N. S.
	10^{-4}	+0.9	3.3380†	0.2696	N. S.
F-O, F-2	10^{-5}	−0.4	0.3008	1.330	N. S.
	10^{-4}	+1.6	1.265	1.265	N. S.
F-O, F-24	10^{-5}	0	0.3203	—	N. S.
	10^{-4}	+6.6	1.108	5.957	§
G-O, G-2	10^{-4}	+7.2	1.082	6.654	§
H-O, H-2	10^{-4}	+6.0	0.8916	6.729	§

* 0, 2, and 24 indicate results 0, 2, and 24 hours after preparation of dilutions.
† Total error includes dilution error.
‡ Not significant.
§ Highly significant ($P<0.01$).

dilution operations, it was necessary to determine the total error ($= \sqrt{(\text{dilution error})^2 + (\text{distribution error})^2}$) before calculating the value of t, and this has been done in table 7.

The dilution error does not enter into comparisons of the counts obtained with the same preparations after standing for various intervals at room temperature, and large discrepancies are apparent in these counts. With 0.1 per cent bovine albumin as diluent, the only significant fall after twenty-four hours was a drop of 32 per cent in the 10^{-4} dilution tube. With both other diluents, however, there were falls of 30 per cent and 31 per cent in two hours at the 10^{-4} dilution, both of which were highly significant, and by twenty-four hours the count had dropped by more than 99 per cent. The superiority of 0.1 per cent bovine albumin solution as a diluent is obvious, especially if dilution tubes are likely to stand for any length of time before use. Schwabacher (27) also found a rapid dying off of tubercle bacilli suspended in distilled water.

Choice of the culture medium: There is no doubt that the most satisfactory method of counting viable bacilli is by the plate count if a suitable medium is available upon which every viable microorganism will develop into a discrete colony.

The advantages of the oleic acid-bovine-albumin-agar medium of Dubos for surface plate counts of *Mycobacteria* may be summarized as follows:

1. The sensitivity of the medium for human-type tubercle bacilli is at least equal to that of any other solid medium and is perhaps greater (28, 29). With bovine-type bacilli it appears to be distinctly superior to other media.
2. Being semi-synthetic it is less liable to suffer from unpredictable variations than egg media.
3. It can be poured in Petri dishes and is transparent, so that examination of plates is easy with either a colony microscope or in a counting box.
4. Colonies of virulent and avirulent strains of mammalian tubercle bacilli develop with their respective characteristic morphologies on this medium.

In their description of the use of the surface drop culture method, Miles and Misra (15) pointed out that the viable count of a very dilute suspension of bacteria will conform to the Poisson series if (a) each plate has an equal chance of receiving any organism and the organisms are distributed independently; (b) the development of any organism is independent of other organisms present, and if development of an organism results in only one visible colony; and (c) each plate offers the same facilities for the development of each organism.

In the present experiments the first requirement is realized by the technique of diluting the mixture and distributing the drops. With tubercle bacilli it is very difficult to satisfy the second condition completely, for even after filtration an appreciable percentage of the bacteria are in clumps of 2 to 5 organisms. Each of the small clumps, like the isolated cells, is capable of giving rise to only one colony. With very dilute suspensions there is no overcrowding in the drop areas. If we count bacterial units, a unit being a single cell or a group up to 8 bacteria, the second condition can be satisfied and approaches the ideal as the proportion of single cells approaches 100 per cent. Fulfillment of the third condition is then a test of the suitability of the nutrient qualities of the medium used, for failure in this would lead not only to a reduction in the number of bacterial units which produced colonies but also in a departure of the count from the ideal Poisson

distribution. This point was directly tested with filtered (58 per cent single cells) and unfiltered (32 per cent single cells) preparations of H37Rv which were inoculated in large series of plates at dilutions calculated from the total counts to give a mean viable count of approximately 1. A comparison of the observed and the expected frequencies in these two series is given in table 8. It was not considered necessary to apply the exact test given by Fisher (30) in these cases.

There is very close agreement between observation and expectation, and for strain H37Rv, at least, it appears that the bacterial units are distributed in the drops of inoculum according to the Poisson distribution and that each bacterial unit gives rise to a colony. This is supported by the rather close correspondence

TABLE 8

Observed and Theoretical Frequency of Viable Tubercle Bacilli (Strain H37Rv) Determined by Surface Plate Counts of Very Dilute Suspensions

PREPARATION	VIABLE COUNT	OBSERVED FREQUENCY fo	EXPECTED FREQUENCY (POISSON DISTRIBUTION) fe	$\frac{(fo-fe)}{fe}$
Filtered culture 1:10^8	0	28	ne^{-m} = 28.13	0.0001
	1	41	ne^{-m} m/1! = 38.35	0.1883
	2	23	ne^{-m} m^2/2! = 26.14	0.3770
	3	10	ne^{-m} m^3/3! = 11.88	0.2980
n^* = 110	4	7	ne^{-m} m^4/4! = 4.05	1.1361
$m\dagger$ = 1.3636	5	1	ne^{-m} m^5/5! = 1.10	
Totals		110	109.65	χ^2 = 1.9995‡
Unfiltered culture	0	30	ne^{-m} = 30.81	0.0213
1:10^7	1	21	ne^{-m} m/1! = 20.54	0.0103
n = 60	2	8	ne^{-m} m^2/2! = 6.85	0.0462
m = 0.667	3	1	ne^{-m} m^3/3! = 1.52	
Totals		60	59.72	χ^2 = 0.0778§

* Number of samples.

† Sample mean.

‡ Table value of $\chi^2_{0.05}$(3df) = 7.815.

§ Table value of $\chi^2_{0.05}$(1df) = 3.841.

observed in each of the 4 experiments with H37Rv between the total and the viable bacterial counts (table 10).

With the filtered preparation of this strain, a comparison was also made between the plate count obtained on oleic acid-albumin-agar (10^{-6} dilution: data shown in table 8) and the number of bottles of liquid Tween-albumin medium which showed growth after inoculation with the same volumes of 10^{-7} and 10^{-6} dilutions of the culture. The results obtained are shown in table 9.

If we apply the formula developed by Halvorson and Ziegler (31) for determining the most probable number of bacteria in each drop of the 10^{-6} dilution, we find that $\hat{x}$ = 1.15, which is in fairly close agreement with the mean figure of 1.36 obtained by the plate count. Davis and Dubos (32) have shown by micromanipulative methods that in the liquid medium a single organism can in all

probability initiate growth, and this experiment indicates that the solid medium gives results at least as good. Espersen's (33) direct microscopic observations showed between 90 and 95 per cent of the bacilli in an 11-day-old culture of human tubercle bacilli in a Tween-albumin liquid medium multiplied when plated on a medium resembling the oleic acid-albumin-agar described in this paper.

All of the experiments with H37Rv indicate the suitability of the solid medium and the satisfactory nature of the technique of dilution and inoculation and of the diluent used.

Tests with Strains Other than H37Rv

The suitability of the diluent, accuracy of the dilution method, and ability of single cells to initiate the development of a colony were studied in detail only with the H37Rv strain of human tubercle bacilli. The results showed that the technique used ensured that the bacterial units were distributed in replicate drops of dilute suspensions according to the Poisson distribution and, further,

TABLE 9

Number of Universal Containers, Each Containing 5 Ml. of Tween-Albumin Liquid Medium Showing Growth Four Weeks After Inoculation with One Drop (Approximately 0.02 Ml.) of Filtered Preparation of H37Rv

DILUTION	NUMBER SHOWING GROWTH	NUMBER NOT SHOWING GROWTH
10^{-7}	20	60
10^{-6}	69	31
10^{-5}*	82	28

* Results with same preparations inoculated with same pipette on oleic acid-albumin-agar solid medium.

that each living bacterial unit, whether it consisted of one bacterial cell or several, was capable of initiating the development of a discrete colony on the surface of the oleic acid-albumin-agar plate.

The same diluent, method of dilution, and method of inoculation of the plates were used with the other strains as had been proved satisfactory with H37Rv. Some idea of the suitability of the medium in each case can be gained by a study of the ratio $\frac{\text{viable count}}{\text{total count}}$, expressed as a percentage in table 10, bearing in mind the relatively high coefficients of variation of both counts. An estimate was made of the reasonable limits of the ratios by determining the standard error of the ratio

$$\left(= \pm \text{ratio} \times \sqrt{\left(\frac{\text{standard error of denominator}}{\text{denominator}}\right)^2 + \left(\frac{\text{standard error of numerator}}{\text{numerator}}\right)^2}\right),$$

and adding or subtracting twice this standard error from the ratio obtained. In determining the ratios $\frac{\text{stained drop count}}{\text{total count}}$ and $\frac{\text{viable count}}{\text{total count}}$, the dilution error was included in the determination of the standard error of the numerators, the

TABLE 10

Comparison of Total and Viable Counts (per ml.) of Tubercle Bacilli

(Figures Refer to Bacterial Units)

GROUP	STRAIN	TREATMENT	TOTAL COUNT—PETROFF-HAUSSER CHAMBER (P.H.C.)		STAINED DROP COUNT, ACID-FAST BACILLI		VIABLE COUNT				RATIOS AND THEIR REASONABLY CERTAIN (TWICE STANDARD ERROR) LIMITS (EXPRESSED AS PERCENTAGES)		
							Dubos medium (D)		Löwenstein medium (L)		Stained drop count / Total count (P.H.C.)	Viable count (D) / Total count (P.H.C.)	Viable count (L) / Viable count (D)
			Mean	S.E.‡	Mean	S.E.‡	Mean	S.E.‡	Mean	S.E.‡			
Human type	H37Rv*	Filtered	4.4×10^7	0.29	3.8×10^7	0.04	3.8×10^7	0.07	—	—	86 (71–101)	86 (62–110)	—
	H37Rv†	Filtered	1.8×10^8	0.20	1.7×10^7	0.16	1.2×10^7	0.04	—	—	94 (65–123)	67 (46–88)	—
	H37Rv	Unfiltered	3.2×10^8	0.28	1.7×10^8	0.17	3.9×10^8	0.36	1.5×10^8	0.32	53 (38–68)	122 (81–163)	38 (21–55)
		Filtered	7.2×10^7	0.28	3.2×10^7	0.24	6.9×10^7	0.28	4.1×10^7	1.68	44 (33–55)	96 (72–120)	59 (12–106)
	H37Ra	Unfiltered	6.6×10^7	0.48	7.0×10^7	0.99	7.2×10^7	0.41	6.3×10^7	1.15	106 (68–144)	109 (77–141)	88 (55–121)
		Filtered	1.3×10^8	0.16	1.9×10^8	0.06	1.3×10^8	0.05	8.6×10^8	1.35	146 (106–186)	100 (66–134)	66 (45–87)
	Amezaga	Unfiltered	1.6×10^8	0.29	1.9×10^8	0.15	1.1×10^8	0.19	2.1×10^7	0.28	119 (69–169)	69 (31–107)	19 (12–26)
		Filtered	2.6×10^7	0.29	2.6×10^7	0.40	1.7×10^7	0.16	1.9×10^6	0.21	100 (59–141)	65 (41–89)	11 (8–14)
	Pratt	Unfiltered	4.7×10^8	0.16	2.9×10^8	0.25	4.4×10^8	0.13	2.7×10^8	0.52	62 (49–75)	94 (71–117)	61 (37–85)
		Filtered	4.2×10^7	0.27	3.0×10^7	0.19	5.4×10^7	0.25	3.1×10^7	0.41	71 (56–86)	129 (93–165)	57 (35–79)
Bovine type	Ravenel	Unfiltered	7.5×10^7	0.42	5.3×10^7	0.12	5.0×10^7	0.17	1.6×10^7	0.17	71 (60–82)	67 (50–84)	32 (25–39)
		Filtered	1.3×10^7	0.08	8.5×10^6	0.44	9.1×10^6	0.52	3.8×10^6	0.48	65 (53–77)	70 (50–90)	42 (30–54)
	Bovine 1	Unfiltered	2.5×10^8	0.23	3.4×10^8	0.06	1.6×10^8	0.10	1.5×10^8	0.10	136 (106–166)	64 (44–84)	94 (76–112)
		Filtered	3.5×10^7	0.20	4.5×10^7	0.11	2.8×10^7	0.10	1.8×10^7	0.20	129 (110–148)	80 (59–101)	64 (50–78)
	BCG	Unfiltered	2.4×10^8	0.16	1.9×10^8	—	1.7×10^8	0.20	1.4×10^8	0.25	79	71 (48–94)	82 (48–116)
		Filtered	9.0×10^8	0.06	6.8×10^8	—	6.4×10^8	0.97	5.5×10^6	0.46	76	71 (44–98)	86 (56–116)
Murine type	OV164	Unfiltered	3.7×10^8	0.26	2.0×10^8	0.25	3.1×10^8	0.09	2.1×10^8	0.56	54 (37–71)	84 (61–107)	68 (32–104)
		Filtered	3.3×10^7	0.22	2.6×10^7	—	2.9×10^7	0.12	1.9×10^7	0.42	80	88 (64–112)	66 (36–96)
M. ulcerans	RT	Unfiltered	1.7×10^8	0.10	—	—	5.1×10^7	0.69	8.3×10^6	3.33	—	30 (19–41)	16 (3–29)
		Filtered	3.9×10^6	0.35	2.5×10^6	—	9.8×10^5	0.35	7.8×10^4	2.22	64	25 (17–33)	8 (3–13)

* Experiment 1 (dilution technique).

† Experiment 2 (choice of diluent).

‡ Standard error of the mean. In each case the standard error = figure shown multiplied by the same power of ten (10^6, 10^5, 10^7, or 10^5) as the mean.

figures used being 5.6 per cent for the stained drop count (dilution to 1:100) and 11.4 per cent for the viable count (dilution to 1:10,000). Since both media were inoculated from the same dilution tube, the dilution error was omitted in comparisons of the viable counts obtained on the Dubos and the Löwenstein media.

With different strains the ratio $\frac{\text{viable count}}{\text{total count}}$ varied between 25 and 129 per cent, but was usually between 70 and 100 per cent, the viable count being that made on the Dubos medium. The important points in the present context are: (a) There is a reasonable consistency between total and viable counts in that the greatest disparity is 75 per cent (with *Mycobacterium ulcerans*) and an unknown proportion of the organisms which failed to produce colonies may not have been viable; and (b) in no instance is there a great discrepancy between the ratios for the unfiltered and filtered preparations of a particular strain in spite of the much higher proportion of isolated bacterial cells in the filtered preparations. These facts suggest that for these other strains also, as for H37Rv, a single bacterial cell was able to initiate the development of a colony.

Two other ratios have been calculated in table 10, allowing a comparison of the suitability of the Dubos and the Löwenstein media and of the results obtained in total counts performed in a Petroff-Hausser chamber and upon stained drops by Crandall's method. The small number of replicate tubes used for the counts on the Löwenstein medium and the variability of the counts made the standard errors of the ratios involving this count very high. Nevertheless, the uniformity of the ratios obtained with filtered and unfiltered preparations of the same strain of bacilli, and the universal failure of the count on the Löwenstein medium to exceed that obtained on the Dubos medium make reasonable the conclusion that the Dubos medium is more satisfactory for the growth of small inocula of all strains of tubercle bacilli. Consideration of table 11 shows also that the optimum time for reading the results was usually several weeks earlier with the Dubos medium.

Crandall's (12) technique of total counts was designed especially for counting dilute suspensions containing between 300,000 and 50,000,000 bacteria per milliliter. This condition held for most of the filtered preparations, and in the later experiments, with greater experience in the measurement of the drops, a close correspondence was obtained between two types of total count.

The actual colony counts of all of the different preparations examined are recorded in table 11. The homogeneity of each set of replicate counts was tested by calculating the index of dispersion (34) $\chi^2 = \frac{\Sigma(x - \bar{x})^2}{\bar{x}}$ for each set, and the results of these calculations are shown in table 11.

For small samples the permissible range of variation of χ^2 is relatively wide, but for samples of 20 or more replications the range is relatively much smaller. If P is the probability of exceeding a given value of χ^2, then a value of P between 0.95 and 0.05 indicates reasonably satisfactory correspondence of the drop count figures with the Poisson distribution.

TABLE 11

Viable Counts of Various Preparations of Tubercle Bacilli, Using Dubos Oleic Acid-Albumin-Agar Medium (D) and Lurie's Modification of the Löwenstein medium (L)

GROUP	STRAIN	TREATMENT	MEDIUM	OPTIMUM TIME FOR READING	DILUTION (X)	DILUTION								OVER-ALL VALUE OF χ^2	
						X				10X					
						Number of replicates	Mean count	Standard error	Index of dispersion χ^2	Number of replicates	Mean count	Standard error	Index of dispersion χ^2	D	L
				weeks											
Human type	H37Rv	Unfiltered	D	3	10^{-6}	20	8.2	0.7540	26.55	15	72.7	4.090	48.33†		
			L	5	10^{-6}	6	3.2	0.6656	4.027		—				
		Filtered	D	3	10^{-6}	110	1.4	0.1138	113.9	40	14.1	0.5681	35.77		
			L	5	10^{-6}	6	0.8	0.3071	3.408	6	5.5	0.8469	4.182	176.22	11.617
	H37Ra	Unfiltered	D	5	10^{-5}	20	15.9	1.013	19.49		—				
			L	5	10^{-5}	6	14.0	1.699	14.43*		—				
		Filtered	D	5	10^{-4}		—			30	24.8	0.9506	26.71		
			L	5	10^{-4}	6	2.2	1.514	11.46*	6	16.5	1.571	12.33*	—	—
	Amezaga	Unfiltered	D	5	10^{-6}	20	2.6	0.4322	27.84	16	24.2	1.333	17.71		
			L	5	10^{-6}	6	0.3	0.1049	1.111*	4	4.8	0.629	1.01		
		Filtered	D	5	10^{-5}	30	3.5	0.3277	26.96	30	35.5	1.482	53.85†		
			L	5	10^{-5}	6	0.3	0.2107	4.034	6	4.0	0.4471	1.499	72.51	6.543
	Pratt	Unfiltered	D	3	10^{-6}	16	8.4	0.6575	12.39	20	93.1	2.860	33.38*		
			L	6	10^{-6}	6	5.7	1.111	6.534	6	34.5	8.955	69.70†		
		Filtered	D	3	10^{-6}	29	11.2	0.5322	20.74	15	105.1	3.406	23.19		
			L	6	10^{-5}	5	12	2.470	12.71*	5	64.8	8.099	23.35	66.32	—
Bovine type	Ravenel	Unfiltered	D	6	10^{-5}	4	12.0	0.913	0.833	19	100.6	3.443	40.3*		
			L	6	10^{-5}	6	5.3	0.4945	1.375	4	31.5	3.476	4.603		
		Filtered	D	5	10^{-4}	20	18.5	1.082	24.05	3	187.3	5.895	1.114		
			L	6	10^{-4}	6	7.7	0.9890	3.826	6	76.8	9.596	3.597	25.99	13.401

Bovine type	Bovine 1	Unfiltered	D	4	10^{-6}	20	2.6	0.3866	21.85	17	32.1	1.977	33.16†		
			L	8	10^{-6}	5	2.2	0.3743	1.274	4	28.5	1.937	1.579		
		Filtered	D	4	10^{-6}	30	3.2	0.3465	32.35	30	58.0	2.039	62.4†		
			L	8	10^{-6}	5	3.2	0.7482	3.499	5	37.4	3.983	8.481	54.20	14.833
	BCG	Unfiltered	D	3	10^{-6}	30	3.2	0.3022	24.59	27	27.3	1.015	26.50		
			L	5	10^{-6}	6	3.0	0.5165	2.667	6	27.7	2.028	4.456		
		Filtered	D	4	10^{-6}	30	1.1	0.1656	22.37	30	11.6	0.5624	23.72		
			L	6	10^{-6}	5	1.0	0.3162	2.000	6	10.8	0.9098	2.291	97.18	11.414
Murine type	OV164	Unfiltered	D	7	10^{-6}	20	5.4	0.3927	10.95	17	64.9	2.038	17.401		
			L	6	10^{-6}	6	4.5	1.205	9.667	6	27.5	6.613	34.97†		
		Filtered	D	7	10^{-6}	10	4.6	0.4763	4.437	26	60.5	2.477	65.96†		
			L	6	10^{-6}	6	3.8	0.8726	5.962	6	38.2	5.206	22.036†	32.79	15.63
M. ulcerans	RT	Unfiltered	D	7	10^{-6}	9	10.3	1.384	13.36	17	89.8	4.075	50.30†		
			L	12	10^{-6}	6	1.7	0.667	8.00	6	15.0	3.088	19.066†		
		Filtered	D	7	10^{-4}	20	1.9	0.2927	17.59	20	18.9	0.6878	9.512*		
			L	12	10^{-4}	6	0.17	—	—	6	1.5	0.4283	3.688	30.95	—

* Significant ($P < 0.05$ or >0.95).
† Highly significant ($P < 0.01$).

If samples with an abnormally large or abnormally small index of dispersion ($P < 0.05$ or > 0.95) are excluded, an over-all χ^2 test can be applied to the remainder since the sum of a number of independent values of χ^2 is itself distributed in the manner shown in the table of χ^2. With each strain studied, the over-all χ^2 value for both the Dubos and the Löwenstein media was determined if there were at least three suitable samples, and its significance was tested by entering the table of χ^2 with a number of degrees of freedom equal to the sum of the degrees of freedom of each set of replicate counts. The results obtained are shown in table 11 (last column). In no case was the over-all value of χ^2 significant.

Before discussing separately the results obtained with the different strains, certain common features deserve comment. First, with a few exceptions the mean counts obtained on the Löwenstein medium were considerably lower than those obtained on Dubos solid medium, and the optimum time for reading was usually some weeks later than the optimum time for reading the oleic acid-albumin-agar plates. The latter were also easier to read as well as to inoculate if several replications were required, but the Löwenstein slopes presented one advantage in their complete freedom from contamination with other micro-organisms, a position which did not invariably obtain with the Dubos plates. Second, when the individual χ^2 values are considered, it is apparent that abnormal variability, exceeding that expected if the counts conformed to the Poisson distribution, was more common at the lower dilution (10X) than at the higher dilution (X) examined. It is probable that this was due to overcrowding which had two possible effects: inhibition of growth of some organisms, and early overgrowth and confluence of colonies which obscured smaller colonies. The impression gained during reading of the plates was that the latter was the more important factor. Third, if the mean counts and their standard errors obtained in the experiments carried out with the dilution "10X" are adjusted by dividing each by 10 and then compared with the mean counts and their standard errors at the dilution "X", a significant difference (determined by the "t" test) is obtained on three occasions out of sixteen pairs of readings (on the Dubos medium). In all three cases (Bovine 1, filtered, and both preparations of OV164), the reading at dilution 10X was appreciably (16 to 45 per cent) higher than the reading obtained at dilution X. No satisfactory explanation can be suggested for these discrepancies from the theoretical tenfold differences. The magnitude of the dilution error between the 10^{-4} and 10^{-5} dilutions is 2.8 per cent and estimation of the total error, including this source of variation, did not greatly affect the value of "t" estimated from the distribution error (standard error of table 11). Nevertheless, the results recorded in table 11 plus those shown in tables 4 and 6 indicate that, in general, a tenfold difference in the dilution tubes was paralleled by tenfold differences in the viable count.

Individual strains may now be discussed separately.

Human-Type Strains

H37Rv: The suitability of the oleic acid-albumin-agar medium for the viable counting of H37Rv has been discussed earlier. The counts obtained on the

Löwenstein medium were approximately half of those obtained on the Dubos medium; and maximal counts were not reached until the fifth week compared with the third week with the Dubos medium.

H37Ra: Tween-albumin cultures of H37Ra invariably showed a greater degree of clumping than was found in cultures of the other mammalian tubercle bacilli, and the figures for the state of the dispersion of H37Ra (table 3) illustrate this difference. The occurrence of these numerous clumps of 5 to 20 bacilli depressed the values of the total and viable counts of H37Ra. Comparison of the total and viable counts, however, showed a satisfactory correspondence between the two sets of figures. With both Dubos and Löwenstein media the optimum time for reading was the fifth week. The internal consistency of the counts on Dubos medium was reasonably satisfactory.

Amezaga: The Dubos medium gave very satisfactory results, viable counts being between 65 and 70 per cent of the total count, and between 5 and 10 times higher than those obtained on the Löwenstein medium. The individual and overall χ^2 tests showed that the distribution of colonies in replicate counts conformed with the Poisson distribution.

Pratt: This freshly isolated human-type strain, which was used in the first subculture after its original isolation on Löwenstein medium, proved a very satisfactory strain for viable counting on the oleic acid-albumin-agar medium. Optimum counts were obtained early (three weeks) and were approximately the same as the total bacterial counts and, except when the counts were large, the distribution of colonies in replicate counts conformed to the Poisson distribution. The Löwenstein medium was somewhat less satisfactory and the optimum counts upon it were not obtained until the sixth week.

Bovine-Type Strains

Ravenel: Counts with the Ravenel strain on the oleic acid-albumin medium were quite satisfactory, reaching 70 per cent of the total count and conforming to the Poisson distribution. The counts obtained on Löwenstein slopes were approximately one-third of those obtained upon Dubos medium.

Bovine 1: This freshly isolated bovine-type strain was used in the first subculture after its isolation on the Löwenstein medium and excellent results were obtained on the oleic acid-albumin-agar medium in the time required for final reading, the approximation of the viable to the total count, and the conformity of the replicate colony counts with the Poisson distribution. The counts upon Löwenstein medium attained almost the same level as those upon Dubos medium, but the colonies were difficult to see and the maximum count was not obtained until the eighth week.

BCG: Preparations of BCG gave approximately the same count in both Dubos and Löwenstein media and the counts were 60 to 70 per cent of the value of the total count. Colonies appeared quite early on Löwenstein medium but the optimum count was not attained until the fifth week, compared with the third week on the Dubos medium. In all instances the internal consistency of the results obtained was satisfactory.

Murine-Type Strain, OV164

The viable count of the vole bacillus on the Dubos medium approximated the total bacterial count, but the maximal count was not attained until the seventh week and even then the colonies were very small. At the higher dilution, the distribution of colonies in replicate counts conformed to the Poisson distribution. On the Löwenstein medium the colonies appeared earlier and reached a larger size, but the maximum count was in all cases somewhat lower than that obtained upon the Dubos medium.

Mycobacterium ulcerans, Strain RT

The viable counts obtained on the Dubos medium were only about 25 to 30 per cent of the total bacterial count. Since the culture examined had been incubated for thirty-two days before use and the culture medium contained glucose,

TABLE 12

Magnitude of Error (Expressed as Coefficients of Variation of the Mean or Percentage Standard Errors) for All Counts Included in Table 10

COUNTING METHOD	DILUTION USED	DISTRIBUTION ERROR		DILUTION ERROR		TOTAL ERROR (AVERAGE)
		Average	Range	Actual (2 per cent)	1 per cent	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
Total—Petroff-Hausser chamber	Undiluted	7.6	0.7-18.1	—	—	7.6
Total—Stained drop	10^{-2}	7.4	1.8-15.3	5.6	2.8	9.3
	10^{-3}			8.4	4.2	11.2
Viable—Dubos medium	10^{-4}	6.6	1.8-17.3	11.4	5.7	13.2
	10^{-5}			14.2	7.1	15.6
Viable—Löwenstein medium	10^{-4}	18.4	6.6-40.1	11.4	5.7	21.6
	10^{-5}			14.2	7.1	23.2

the percentage of viable organisms may have been quite low. Dubos and Fenner (10) found that, if BCG was incubated in the Tween-albumin medium containing glucose, the viable count dropped appreciably during the third and fourth weeks, and the same effect may have been operative here. The fall did not occur in the absence of glucose, but shorter periods of incubation or the omission of glucose from the medium resulted in poor growth of all strains of *Mycobacterium ulcerans*. As is usual with this organism, the count did not reach its maximum level until the seventh week of incubation at 33°C. On the Löwenstein medium the colonies appeared later still and the optimum count was not obtained until the twelfth week. Except for one count which showed less variability than expected by the Poisson distribution, counts made of reasonable numbers of colonies conformed to this distribution.

DISCUSSION

The errors involved in counting *Mycobacteria* arise from several sources. *Mycobacteria* grow in clumps or cords even in Tween-albumin media, and counts

therefore record only the number of bacterial units or groups, rather than the number of individual bacilli. If a differential count is made of the state of dispersion of the bacteria, the total number of individual bacilli can be estimated. In view of recent observations on the multinucleate nature of tubercle bacilli, particularly the young organisms (35 and 36), the question of what constitutes an individual microorganism may need to be reconsidered. In the present paper all counts refer to bacterial units although an attempt was made to estimate the number of single acid-fast bacilli and groups of two, three, four, and five or more acid-fast bacilli in each preparation.

An error which has been ignored by most bacteriologists is that involved in the preparation of the dilutions used for the different types of count, the dilution error (17). This error increases with the number of steps necessary to obtain the dilution used for making the count and is, therefore, much greater in viable counts than in counts in the Petroff-Hausser chamber, in which no dilution operations are necessary. A small dilution error was involved in preparation of material for the stained drop counts for, with the cultures used, dilutions of 1:100 or 1:1,000 were required to obtain suitable numbers of organisms in the small drops. Jennison and Wadsworth (17) consider that with well-graduated pipettes and careful technique the error of delivery from one-milliliter and ten-milliliter pipettes may be as low as 1 per cent, but tests of the pipettes and technique used in the present investigations showed that this error was 2 per cent. From the table prepared by Jennison and Wadsworth it is possible to calculate the total error (as a percentage standard error) involved in the preparation of any particular dilution. Average figures for these errors are included in table 12. For the viable counts, four or five dilution steps, consisting of the delivery of 1.0 ml. into 9.0 ml. blanks, were necessary to obtain the correct dilution for plating. Examination of table 12 shows that for these counts the error due to the dilution process was of considerable magnitude compared with the distribution error and, even if pipettes and technique were changed to reduce the standard errors of pipettes and blanks to 1 per cent, the dilution error is about the same as the distribution error. Added to this are such factors as adequate mixing of the suspensions, possible breakdown of clumps during the dilution operations, and the use of a diluent in which maximum survival of the viable organisms is ensured. It is clear that, if a satisfactory medium is available, the process of obtaining the dilutions required for inoculation of the plates is the step upon which attention should be concentrated if accurate viable counts are required.

The error to which most consideration has been given is the distribution error, and in all types of counts the distribution should conform with the Poisson series. Direct test with one strain of tubercle bacilli, H37Rv, showed that the distribution of colonies on the surface of oleic acid-albumin-agar medium from a large number of replicate drops of a very dilute suspension did conform with the Poisson distribution. In the other experiments, the distribution error was determined by calculating the standard error of the mean counts.

In table 12 the average errors, expressed as coefficients of variation of the mean, are shown for all the counting methods employed in all of the experiments included in table 10.

The magnitude of the errors involved in counting bacteria should be fully appreciated before attaching too much significance to variations of less than 20 per cent between replicate viable counts. Total (group or unit) counts are somewhat more accurate.

The results obtained in the present experiments amplify considerably those previously reported (6) and show that, by the use of the surface drop counting method on the oleic acid-albumin-agar medium of Dubos, relatively accurate viable counts can be obtained, not only with old laboratory strains like H37Rv but also with freshly isolated bovine- and human-type strains, with the vole bacillus, and with *Mycobacterium ulcerans*. Moreover, comparison of total and viable counts shows that with the great majority of strains the ratio of viable count to total count is high and for any particular strain is not reduced by filtration of the specimen, which greatly increases the proportion of isolated bacterial cells. It is likely, therefore, that the majority of organisms in the Tween-albumin liquid medium are viable and that all viable organisms, or groups of organisms, initiate the development of a colony on the oleic acid-albumin-agar medium. These results confirm those of Espersen (33) and differ from the observations recorded by Wilson and Schwabacher (37) who found that only a small proportion of the bacteria removed from the surface of a solid egg medium and dispersed by gentle grinding were viable.

The importance of using a satisfactory diluent in preparing the dilutions used for the viable counts must be emphasized. The superiority of a 0.1 per cent solution of bovine albumin was amply demonstrated by the experiment with H37Rv, and other strains have proved still more susceptible to noxious environmental influences, against which they can be protected by bovine albumin. Lurie and associates (37a) have recently reported that the viability of tubercle bacilli atomized in the apparatus used for quantitative airborne infection of rabbits is considerably enhanced when the organisms are suspended in 0.1 per cent bovine albumin solution instead of M/15 disodium hydrogen phosphate.

Studies at present in progress suggest that there may be a high and relatively constant ratio between total and viable bacteria in young cultures of virulent tubercle bacilli grown in Tween-albumin liquid medium. If this proves to be so, the simple performance of a total bacterial count at the time of inoculation of animals may permit a rapid and accurate estimate to be made of the number of viable bacilli inoculated.

Consideration of data based upon the theoretical distribution of bacteria in aliquots of a bacterial suspension (table 1) indicates that the larger the number of bacterial colonies counted in each replicate drop, the greater the opportunity of detecting differences between comparable suspensions, using a given number of replications. Against this requirement must be set the necessity for avoiding overcrowding or confluence in drop areas 10 to 15 mm. in diameter. With most strains, approximately 50 colonies per drop was the largest number which could be counted with ease although with the organisms like the murine-type bacillus

and H37Ra, which produced much smaller colonies, accurate counts could be made of more than 100 colonies per drop area.

A disadvantage of the Dubos solid medium dispensed in Petri dishes is its suitability for the growth of molds and fungi. Dyes which inhibit the development of fungi are also slightly inhibitory to tubercle bacilli, but the incorporation of actidone (38) or penicillin and grisein (39) may satisfactorily inhibit the development of such contaminants without affecting the development of tubercle bacilli.

In all of the experiments recorded, the oleic acid-albumin-agar medium of Dubos proved superior, both in rate of growth of the colonies and in the counts finally attained, to Lurie's modification of the Löwenstein medium. Nevertheless, the results obtained with the latter medium were rarely less than 50 per cent of those obtained on the Dubos medium. Such a difference would not affect greatly the significance of the counts made over many years by Lurie and his collaborators. It should be pointed out, however, that for the counts recorded here only culture suspensions were used. Many other problems, such as satisfactory selection of samples from organs and the toxic effects of constituents of animal tissues, must be studied before proper assessment is possible of viable counts made on suspensions of the tissues of infected animals.

The existence of such a simple and accurate method of counting viable tubercle bacilli as is here described should render fruitful a reinvestigation of many of the problems of the pathogenesis of tuberculosis. It should also allow a final discard of the inaccurate and misleading habit of describing doses of living tubercle bacilli in terms of the weight or volume of the bacillary mass injected.

SUMMARY

A detailed description is given of the technique of performing viable counts of tubercle bacilli by the surface drop method on the oleic acid-albumin-agar medium of Dubos.

With each of several strains tested, including recently isolated as well as standard laboratory strains and representatives of all types of mammalian tubercle bacilli, the results indicated that each viable bacterium would initiate the development of a colony. Direct comparison showed that the Dubos oleic acid-albumin-agar medium was more satisfactory for these counts than Lurie's modification of the Löwenstein medium.

With the majority of strains a high percentage (70 to 100 per cent) of the bacilli grown in the Tween-albumin liquid medium were viable as judged by their ability to produce a colony upon the oleic acid-albumin-agar medium.

The importance of the selection of the diluent is illustrated by an experiment in which several diluents were compared, and the superiority over other fluids of a 0.1 per cent dilution of bovine albumin in water was obvious.

The dilutions to be inoculated should be chosen so that one of those tested will yield 40 to 50 colonies in each drop area produced by a drop of 0.02 ml. volume.

SUMARIO

La Enumeración de los Bacilos Tuberculosos Viables por Cuentas Superficiales en las Placas

Descríbese con todo pormenor la técnica de ejecutar numeraciones de los bacilos tuberculosos viables por el método de la gota superficial en el medio de agar-albúmina-ácido oleico de Dubos.

Con cada una de varias cepas estudiadas, comprendiendo cepas recién aisladas así como las corrientes del laboratorio y representantes de todas las formas de bacilos tuberculosos de mamíferos, los resultados indicaron que cada bacteria viable iniciaría la formación de una colonia. La comparación directa demostró que el medio de agar-albúmina-ácido oleico de Dubos era más satisfactorio para dichas numeraciones que la modificación por Lurie del medio de Lowenstein.

Con la mayoría de las cepas, un alto porcentaje (70 a 100) de los bacilos cultivados en el medio líquido de albúmina-Tween fué viable, a juzgar por su capacidad para producir colonias en el medio de agar-albúmina-ácido oleico.

La importancia de la selección del diluente fué ilustrada por un experimento en el cual se compararon varios diluentes, y resultó manifiesta la superioridad sobre otros líquidos de una dilución acuosa de 0.1 por ciento de albúmina bovina.

Hay que escoger las diluciones por inocular de modo que una de las ensayadas rinda entre 40 y 50 colonias en cada zona formada por una gota de 0.02 ml. de volumen.

RESUME

La classification des bacilles tuberculeux actifs à l'aide de la botte de Pétri

Description détaillée de la méthode de numération des bacilles tuberculeux actifs par l'ensemencement en goutte et en surface sur milieu de Dubos (acide oléique-albumine-agar).

L'expérimentation faite à l'aide de plusieurs souches connues comprenant de nouvelles souches récemment isolées ainsi que des souches standard et représentatives de tous les types de bacilles tuberculeux de mammifère, indique que chaque bacille actif serait le point de départ d'une colonie. La comparaison directe montre que le milieu de Dubos (acide oléique-albumine-agar) est plus satisfaisant pour ce test que le milieu de Löwenstein modifié par Lurie.

Pour la majorité des souches, un haut pourcentage (70 à 100%) des bacilles cultivés dans le milieu liquide Tween-albumin était actif, comme le montre la production d'une colonie sur le milieu de Dubos. L'importance de la sélection du substrat est démontrée par une expérience où on a comparé leur différentes valeurs et la supériorité d'une dilution d'albumine de boeuf à 1% en milieu aqueux. La dilution à innoculer doit être choisie de manière à ce que 40 à 50 colonies poussent sur chaque surface ensemencée par une goutte de 0.02 mm³.

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A STUDY OF THE RELATION OF NUTRITION TO THE DEVELOPMENT OF TUBERCULOSIS[1,2]

Influence of Ascorbic Acid and Vitamin A

HORACE R. GETZ,[3] ESMOND R. LONG, AND HOWARD J. HENDERSON

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INTRODUCTION

Observations on the relation of nutrition to tuberculosis are scattered through the medical literature of the last thirty years. The various studies reported include experimental investigations on animals and observations in man, particularly with respect to vitamin intake and vitamin levels during the course of the disease. Reviews by Perla and Marmorston (1, 2), Sweany and his colleagues (3), and Long (4) may be consulted for observations on various aspects of the subject up to 1940.

Attention was drawn to a favorable effect of vitamin C by McConkey and Smith (5), and to deficiency in vitamin A in tuberculous patients by Crimm and Short (6) and McConkey (7). Numerous investigators have described a depressing effect of vitamin C on tuberculin sensitivity. In general, the evidence presented indicates that animals deficient in vitamin C have less resistance than normal to tuberculosis and that the tissues of human beings with tuberculosis have subnormal levels of this vitamin and vitamin A.

For a review of more recent literature, readers are referred to an extensive review of the relations of tuberculosis and nutrition in *The Biology of Human Starvation* by Keys, Brozek, Henschel, Mickelson, and Taylor (8). This review includes a bibliography on observations of the prevalence of tuberculosis in malnourished populations and calls attention to the pitfalls in casual use of such data, when many factors other than nutrition itself may be involved. Much has been written on the prevalence of tuberculosis in large populations subjected to excessive hardships in war and disaster. Sharp rises in tuberculosis occurred throughout most of Europe during both world wars, and numerous observers attributed the rise in large part to malnutrition although all admitted that other factors played an important role (see, for example, Hoefnagels (9) on tuberculosis in Holland during World War II).

Such studies deal with the rise and fall of tuberculosis in large populations without reference to individuals. Few investigations have been made of the relation of nutrition and tuberculosis in which accurate data were available on nutrition and tuberculosis in groups of persons followed individually over a suitable period of time. One such study has been reported recently, however, which is

[1] From the Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pennsylvania.

[2] Aided by a grant from the John and Mary R. Markle Foundation.

[3] At present, Medical Director, The Charles Cook Hastings Home, an institution for the study and treatment of tuberculosis, Altadena, California.

therefore of unusual interest. In this study Downes (10) reported a difference in the incidence of tuberculosis in a Negro population in New York which was followed for a period of three years. The population was divided into three groups. Vitamin and mineral supplements were given to two of the groups, while the third served as a control. Ten cases of tuberculosis developed in the control group and only one in the groups which took the supplements. Three more cases developed in those who started taking the supplements but did not continue. Thus, the incidence of new cases of tuberculosis was appreciably lower among those taking vitamins than in control families. Miss Downes stated, however, that the difference did not have a probability below the 0.05 generally used as a limit for significance.

In the study which forms the basis for the present report, no nutritional therapy was attempted. The nutritive level in each subject was followed to learn if any significance could be attached to low nutritive levels in regard to the development of tuberculosis in an untreated population.

OBSERVATIONS

Plan of Study

The group subjected to study consisted of a population diagnosed as nontuberculous at the beginning of the investigation, in which objective nutritional tests were made before and throughout the period of observation. In an earlier search to determine the prevalence and extent of malnutrition in tuberculous persons (11, 12, 13) some nontuberculous persons were examined. These persons lived in a low-income area in Philadelphia where both malnutrition and tuberculosis exist and where exposure to tuberculosis is probable. Forty-three per cent of them had known contact with active tuberculosis (table 1). With this material already available, it was thought wise to expand the group and study a larger number over a period of years to learn if a low level of any one nutrient predisposed to tuberculosis. In order to make the basic material as nearly homogeneous as possible, it was decided to limit it to one sex, one race, and one age span. Negroes exceeded white persons in number in the clinic and were chosen as likely to yield a larger number of cases of tuberculosis for study. The selection of males eliminated periodic physiologic changes which occur in women. No significant physiologic variation occurs in males of the early and middle adult years. It was finally decided, therefore, to restrict new selections for study to Negro males between 20 and 45 years of age. This report covers seven years of study; the last five and one-half years were directed exclusively toward obtaining information on the nutritional factors involved in the first development of tuberculosis.

At the beginning of the study it seemed possible to have 500 nontuberculous males under observation at all times. This number was considered large enough to serve as a representative sample, even though some of the 500 subjects were lost and replaced by new subjects. During the entire investigation a total of 1,100 males, aged 20 to 45 years inclusive, were included. Eighty-three per cent were Negroes, and the rest white. Six per cent of the subjects were receiving

financial help from the Department of Public Assistance. Fifty per cent came to the clinic without breakfast, 63 per cent stated they had less than one pint of milk a day, and 16 per cent were taking cod-liver oil or some vitamin supplement.

A detailed nutritional examination, as well as a complete clinical examination, was made on the first visit to the clinic, which included calculation of per cent standard weight and measurement of erythrocyte sedimentation rate, blood hemoglobin, plasma carotene, vitamin A and vitamin C, total serum protein, serum albumin and globulin and serum calcium, phosphorus, and phosphatase. Bone density studies also were made on a large proportion of these subjects. The plan was to re-examine all subjects by chest roentgenography and to re-evaluate their nutritional status every six months. As the study actually progressed many patients came to the clinic more often than this, and some only

TABLE 1

All Nontuberculous Subjects Included in Study and Those Who Later Developed Tuberculosis, Classified by Extent of Contact

EXTENT OF CONTACT	NONTUBERCULOUS SUBJECTS		NONTUBERCULOUS SUBJECTS WHO LATER DEVELOPED TUBERCULOSIS	
	Number	Per cent distribution	Number	Per cent distribution
Total	1,100	100.0	28	100.0
No contact known or admitted	628	57.1	17	60.7
Contact with sputum-"positive" or probably "positive" cases	410	37.3	7	25.0
Contact with sputum-"negative" or probably "negative" cases	62	5.6	4	14.3

after longer periods of time. The first patients admitted to the study entered during World War II; these were fluoroscoped but not examined by chest roentgenograms, as films were in short supply at the time. This was a mistake, as was later learned, and, when films were available, roentgenographic examinations were made on all subjects at the time of admission to the study. Adequate clinical examination supplemented roentgenographic examination on each visit.

Stability of Nutritional Status

After the study had been in progress five years, stability tests were made on the observations to establish the limits of variation of each of the nutritional components. First, the results obtained by the various nutritional tests were transferred to punch cards and analyzed by years to determine whether there had been any change in the sampling of the population, change in the population itself, or change in procedure that might have affected the data. Frequency distribution curves were plotted for each of the nutritional components for each year. There was some variation, but the curves were judged not to have diverged significantly.

The frequency distribution curves for the second test showed a slight shift toward a higher mean value than that found in the first-test curves. The third-test curves showed a still further shift toward a higher nutritive level. Table 2 gives the arithmetic means for five of the blood nutrient components. It is believed that this indicates that those returning for a second test and those returning for a third test represented a selected group in which the nutritive level in general was better than that of the mass population, as sampled by the first testing. The data obtained from the first testing were judged to be appropriate for the purposes of the study as they included all 1,100 subjects and there was no selection. The first-test data gave nutritive levels at the time nearest the known exposure to tuberculosis.

An analysis was made of the stability of the blood nutrient levels in the persons with the highest values. In a previous paper the nutrient levels observed in the blood were designated on apparently appropriate bases as normal, borderline, and pathologically deficient. This division was made in order to show per-

TABLE 2

Arithmetic Means of Test Values for First-, Second-, and Third-Test Data, Classified by Blood Nutrient Component

BLOOD NUTRIENT COMPONENT	ARITHMETIC MEANS		
	First test	Second test	Third test
Vitamin A—I.U. per 100 ml	100.0	108.5	112.5
Vitamin C—mg. per 100 ml	0.10	0.11	0.14
Hemoglobin—gm. per 100 ml	15.0	15.3	15.2
Albumin—gm. per 100 ml	4.5	4.6	4.7
Calcium—mg. per 100 ml	10.0	10.3	10.5

centage variations in the nutrient test values of tuberculous and nontuberculous subjects. The same division of the test values has been used in analysis of the data in the present report, as it is believed that the divisions are essentially correct. Moreover, even if not wholly justified for all purposes, division of the nutritive levels into these three groups permits easy comparison of the findings. With respect to vitamin A, 65 per cent of the original "normal" group when retested were still "normal" (see table 3 for variations with the lapse of time between the first and second tests). With respect to vitamin C, the figures are even more striking, as most of the subjects had levels in the pathologically deficient group; figures are given in table 3 for this group. Table 3 also includes the values for hemoglobin and albumin. The values for these four nutrient components indicate that the nutritive level was approximately constant, even when observed over a long period of time.

Methods Used for Nutritional Tests

Methods used for the nutritional tests were the same as those reported in the previous papers (12, 13). Macroscopic methods were used for the chemical determinations of both vitamins A and C.

Status in Respect to Tuberculosis

The study was started in 1942 while the prevalence of malnutrition in tuberculous subjects was being investigated and ran through June 1949. It was recognized that the population group selected could not have the same incidence of tuberculosis as a previously unexposed population, but by running the study over a number of years, with a large number of subjects in the study at all times, it was believed that enough cases of tuberculosis would develop so that the findings would be of significance. Great care was exercised to see that continuity of techniques was maintained, and personnel changes were kept as low as possible during the study.

In order to study the data statistically, definite limitations were used for determining the length of time a person was observed as nontuberculous in the study. If the person was seen only once, he was counted as being nontuberculous and in the study for only one month. If he was seen twice and was nontuberculous

TABLE 3

Percentage of Men Originally with "Normal" Blood Levels Whose Retest Values Were in the Same Group After Specified Periods, Classified by Blood Nutrient Component

BLOOD NUTRIENT COMPONENT	PERCENTAGE OF SUBJECTS ORIGINALLY WITH "NORMAL" BLOOD LEVELS WHOSE RETEST VALUES WERE IN THE SAME GROUP AFTER SPECIFIED PERIODS				
	Less than 7 months	7 to 13 months	13 to 25 months	25 to 37 months	37 months or more
Vitamin A	56	66	72	81	79
Vitamin C*	90	90	87	86	100
Hemoglobin	59	65	79	80	73
Albumin	77	78	81	85	87

* Percentages are for those in deficient group as they were the largest group.

on both examinations, he was considered to have been nontuberculous in the interim. We feel certain that a great many of the subjects who were seen only once continued to be nontuberculous, but they were not counted as nontuberculous for more than one month unless they were re-examined in the clinic and a chest roentgenographic examination was made. Considerable effort was expended to bring all of the subjects back for re-examination. The voting register for the city of Philadelphia was checked to obtain knowledge of deaths and new addresses. The Department of Public Assistance also supplied information which helped us to bring some members of the group back to the clinic. Special permission was obtained to return those patients attending other hospitals and clinics so that we might have follow-up examinations. Hospital autopsy records were also willingly supplied to help this study. The war affected the number of nontuberculous males in the population, but the clinic attendance was not seriously curtailed for this study until a few months before the war ended.

Results

Twenty-eight men, first diagnosed as nontuberculous, were later diagnosed as tuberculous. In 16 cases the lesions discovered proved to be active and in 12

cases the lesions appeared by roentgenographic examination to be stable when first seen. In none of the 12 lesions has clinical activity developed since the original detection although in 4 cases the lesions shrank slightly or became more fibrous. Since these lesions were fluctuating, even though in the direction of arrest, they had presumably been active recently. Ten of the patients with inactive lesions were originally declared nontuberculous on the basis of fluoroscopy only, and it is possible that the lesions seen later on roentgenographic examination were present when the patients were first examined. Two of the 12 patients with inactive lesions were examined originally by chest roentgenography and declared negative for tuberculosis.

It appears highly improbable that the development of tuberculosis in these 28 men can be explained on the basis of greater exposure to tuberculosis than in

TABLE 4

Subjects with Specified Case Numbers Who Were Negative by Roentgenographic Examination at the Beginning of the Study and Positive by Roentgenographic Examination Later (Group I), Classified by Per Cent of Standard Weight and Blood Component

PER CENT OF STANDARD WEIGHT AND BLOOD COMPONENT	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5	CASE 6	CASE 7	CASE 8
Per cent of standard weight.....	98	82	97	93	110	102	100	88
Blood component:								
Hemoglobin—gm. per 100 ml.....	16.0	13.6	12.9	15.9	15.7	16.7	14.7	15.9
Vitamin A—I.U. per 100 ml.....	55.0	81.0	81.9	104.0	63.0	32.0	121.0	67.0
Carotene—mg. per 100 ml.....	0.07	0.11	0.04	0.09	0.06	0.11	0.10	0.06
Vitamin C—mg. per 100 ml.....	0.08	0.02	0.00	0.39	0.02	0.00	0.00	0.00
Albumin—gm. per 100 ml.....	4.0	4.3	4.0	4.6	4.4	4.6	3.7	4.1
Globulin—gm. per 100 ml.....	2.8	2.2	2.8	3.0	3.4	3.0	2.3	2.9
Calcium—mg. per 100 ml.....	—	10.2	9.0	11.5	10.9	10.7	9.6	13.5
Phosphorus—mg. per 100 ml.....	4.8	4.5	3.6	3.9	—	2.7	3.5	2.9
Phosphatase—mg. per 100 ml.....	1.9	2.0	2.6	1.6	—	3.1	3.2	1.6

those not developing tuberculosis. As table 1 shows, known contact with "sputum-positive" cases of tuberculosis was actually less frequent in those who developed tuberculosis than in those who remained nontuberculous.

For comparative purposes, the 28 subjects who developed tuberculosis were divided into three groups. *Group I* is composed of persons who were negative by chest roentgenography on admission to the investigation and positive later by chest roentgenography. All of these had clinically active disease. *Group II* is made up of those who were considered to be nontuberculous on fluoroscopic examination and later proved positive for tuberculosis by chest roentgenography. All of these also had clinically active disease. *Group III* comprises those subjects who were negative for tuberculosis by original chest roentgenography or fluoroscopy and positive later, but with nonclinical tuberculosis as shown by chest roentgenography and clinical study.

The findings on all of these subjects are presented in tables 4, 5, and 6. The data in tables 4 and 5 are so nearly alike that the patients in *Group I* and *Group*

II may be considered as essentially the same group. Both groups had active tuberculosis. In *Group I*, 3 had minimal pulmonary tuberculosis, one of them with tuberculous osteomyelitis; 3 had moderately advanced pulmonary disease; one had far advanced pulmonary disease; and one had no demonstrable pulmonary tuberculosis but died of tuberculous peritonitis. In *Group II*, 4 had

TABLE 5

Subjects with Specified Case Numbers Who Were Negative by Fluoroscopy at the Beginning of the Study and Positive by Roentgenographic Examination Later (Group II), Classified by Per Cent of Standard Weight and Blood Component

PER CENT OF STANDARD WEIGHT AND BLOOD COMPONENT	CASE 9	CASE 10	CASE 11	CASE 12	CASE 13	CASE 14	CASE 15	CASE 16
Per cent of standard weight	110	83	96	99	95	94	99	91
Blood component:								
Hemoglobin—gm. per 100 ml.	—	15.2	15.1	15.0	12.9	14.3	15.7	16.3
Vitamin A—I.U. per 100 ml.	49.1	75.4	81.0	73.1	61.0	53.0	133.0	66.7
Carotene—mg. per 100 ml.	0.06	0.12	0.05	0.10	0.08	0.09	0.06	0.06
Vitamin C—mg. per 100 ml.	0.21	0.00	0.00	0.00	0.00	0.00	0.00	—
Albumin—gm. per 100 ml.	—	3.8	4.1	4.5	4.3	4.6	4.1	4.1
Globulin—gm. per 100 ml.	—	3.6	2.1	1.5	3.1	2.6	3.6	4.1
Calcium—mg. per 100 ml.	—	—	9.8	9.4	9.2	10.3	9.2	—
Phosphorus—mg. per 100 ml.	—	—	3.1	3.3	3.4	4.1	2.9	3.7
Phosphatase—mg. per 100 ml.	—	—	1.6	1.7	1.3	1.2	2.9	1.3

TABLE 6

Subjects with Specified Case Numbers Who Were Negative by Roentgenographic Examination or Fluoroscopy at the Beginning of the Study and Positive Later but With Nonclinical Tuberculosis (Group III), Classified by Per Cent of Standard Weight and Blood Component

PER CENT OF STANDARD WEIGHT AND BLOOD COMPONENT	CASE 17	CASE 18	CASE 19	CASE 20	CASE 21	CASE 22	CASE 23	CASE 24	CASE 25	CASE 26	CASE 27	CASE 28
Per cent of standard weight	96	87	87	84	110	106	113	82	109	86	97	94
Blood component:												
Hemoglobin—Gm. per 100 ml.	14.1	14.6	14.7	14.5	16.2	14.1	17.0	13.6	15.3	15.1	14.6	15.1
Vitamin A—I.U. per 100 ml.	135.4	74.8	93.6	84.0	86.0	80.0	102.0	122.0	129.1	154.0	88.8	74.8
Carotene—mg. per 100 ml.	0.08	0.11	0.13	0.11	0.08	0.06	0.06	0.08	0.09	0.12	0.07	0.07
Vitamin C—mg. per 100 ml.	0.46	0.49	0.19	—	0.03	0.16	0.57	0.16	0.03	0.00	0.48	0.00
Albumin—gm. per 100 ml.	4.9	3.9	3.9	3.8	4.7	3.6	4.5	4.6	5.4	4.5	3.9	4.6
Globulin—gm. per 100 ml.	2.4	3.2	2.9	3.6	2.6	2.1	2.5	3.2	2.6	3.3	2.8	1.9
Calcium—mg. per 100 ml.	9.6	9.8	9.3	9.6	10.0	8.7	9.4	10.5	10.1	10.8	9.6	9.0
Phosphorus—mg. per 100 ml.	3.8	4.4	3.9	3.8	3.0	3.1	3.7	3.4	2.6	3.4	3.1	3.9
Phosphatase—mg. per 100 ml.	1.5	2.0	1.9	1.9	2.6	2.1	1.9	2.7	2.5	3.9	3.3	3.0

minimal pulmonary tuberculosis, one of them with osteomyelitis; 2 had moderately advanced pulmonary disease; and 2 had far advanced disease. In *Group III*, 10 had minimal pulmonary tuberculosis; 2 had moderately advanced pulmonary disease; and none had far advanced disease.

When the first-test data for the subjects who later developed tuberculosis are spotted on first-test frequency distribution curves for the 1,100 subjects tested, it is found that the observed values are scattered all through the frequency dis-

tribution curves for all of the tests made except the test values of vitamins A and C. This is apparent in the data in tables 4 and 5. It is believed that this indicates no correlation between the nutritive level of these components and the development of tuberculosis. The two exceptions, viz., vitamins A and C, are believed important as the observed values for the subjects who later developed active tuberculosis are all at the lower end of the range of values for all 1,100 subjects.

TABLE 7

Subjects Who Remained Nontuberculous and Those Who Developed Tuberculosis, Classified by Plasma Level of Vitamin A

INTERNATIONAL UNITS OF VITAMIN A PER 100 ML. OF PLASMA	SUBJECTS WHO REMAINED NONTUBERCULOUS		SUBJECTS WHO DEVELOPED TUBERCULOSIS					
			Group I		Group II		Group III	
	Number	Per cent distribution	Number	Per cent distribution	Number	Per cent distribution	Number	Per cent distribution
Total	1,058	100.0	8	100.0	8	100.0	11	100.0
Less than 85	318	30.0	6	75.0	7	87.5	3	27.3
85 to 110	292	27.6	1	12.5	—	—	4	36.4
110 or more	448	42.4	1	12.5	1	12.5	4	36.4

TABLE 8

Subjects Who Remained Nontuberculous and Those Who Developed Tuberculosis, Classified by Plasma Level of Vitamin C

MILLIGRAM PER ML. OF PLASMA VITAMIN C	SUBJECTS WHO REMAINED NONTUBERCULOUS		SUBJECTS WHO DEVELOPED TUBERCULOSIS					
			Group I		Group II		Group III	
	Number	Per cent distribution	Number	Per cent distribution	Number	Per cent distribution	Number	Per cent distribution
Total	1,013	100.0	8	100.0	7	100.0	12	100.0
None	381	37.6	4	50.0	6	85.7	3	25.0
Less than 0.60	515	50.8	4	50.0	1	14.3	9	75.0
0.60 or more	117	11.6	—	—	—	—	—	—

Another computation was made by comparing the proportion of the subjects in each nutritive level, i.e., those that later developed tuberculosis, with the whole group studied as nontuberculous. Again, only the values for vitamins A and C give findings that are significant. They are given in tables 7 and 8. It is to be noted that more of the subjects who developed active tuberculosis (*Groups I* and *II*) had a low level of vitamin A than the nontuberculous population as a whole. Likewise, *all* of those who developed tuberculosis had a low level of vitamin C.

The data for *Groups I* and *II* in table 7 were tested against the data of the

nontuberculous group before the percentages were calculated. Using the chi-square formula, the probability was less than 0.01; that is, there is one chance in 100 that these figures could occur by chance distribution. Therefore, the difference is highly significant. *Group III* data in table 7 were indistinguishable from the nontuberculous group when similarly tested. In table 8, the chi-square formula was likewise applied and *Groups I* and *II* differed from the nontuberculous group significantly; the probability was less than 0.05. If 4 more subjects had similarly fallen into *Groups I* and *II*, the data would have been highly significant. *Group III* data in table 8, when tested against the data of the nontuberculous

TABLE 9

Number of Subjects Observed and Number of Person Observation Years, Classified by Year

YEAR	NUMBER OF SUBJECTS OBSERVED	PERSON OBSERVATION YEARS
1942 to 1949	1,100	1,563.57
1942	202	71.50
1943	258	169.25
1944	317	243.66
1945	337	248.00
1946	402	257.08
1947	455	288.58
1948	454	236.17
1949	160	49.33

TABLE 10

Person Observation Years Per Case of Tuberculosis, Classified by Plasma Level of Vitamin A

INTERNATIONAL UNITS OF VITAMIN A PER 100 ML. OF PLASMA	PERSON OBSERVATION YEARS PER CASE OF TUBERCULOSIS
Less than 85	31.11
85 or more	90.32

group, are less significant; the probability was less than 0.20. *Group III*-vitamin C data are not greatly different from the data of the nontuberculous group.

The person observation years were computed to learn the total for the study and for each year. Data are given in table 9. When the number of person observation years necessary to find a case of tuberculosis is determined for the nutrient components, again only the data for vitamins A and C are significant. They are presented in tables 10 and 11. Those that had low levels of vitamin A clearly yielded the greatest number of cases of tuberculosis, and, as noted in table 11, those with low levels of vitamin C yielded *all* of the 28 cases of tuberculosis.

In a few of the subjects who developed tuberculosis, bone density determinations were made. The results showed no significant variation from the findings on the whole nontuberculous population.

Those 156 nontuberculous subjects who took cod-liver oil or vitamins had

somewhat better values of vitamins A and C than the population as a whole. In 4 of them, lesions were detected in the course of the study, viz., 2 in *Group II* and 2 in *Group III*.

Deaths

During the seven years of the study, in which 1,100 subjects were included, there were 16 known deaths, 4 of which were due to tuberculosis (*Group I* and *Group II* patients). Three subjects died of pneumonia, 4 of cancer, one of heart disease, one of diabetes, and 2 of unknown causes. The test values for the persons who died of the various diseases listed showed no tendency to group on the frequency distribution curves, except for those in which death was due to tuberculosis.

TABLE 11

Person Observation Years Per Case of Tuberculosis, Classified by Plasma Level of Vitamin C

MILLIGRAM PER ML. OF PLASMA VITAMIN C	PERSON OBSERVATION YEARS PER CASE OF TUBERCULOSIS
Less than 0.6 mg. per 100 ml	45.76*
0.6 or more mg. per 100 ml	—†

* All 28 cases were in this group.

† No case discovered in 188.42 person observation years.

SUMMARY

A group of 1,100 men, free from pulmonary tuberculosis at first examination as shown by roentgenographic study, were followed over periods ranging from one month to five years. The average number of years of observation per person was 1.5. Eighty-three per cent of the group were Negroes and 17 per cent white. All were between 20 and 45 years of age. Periodic roentgenographic examinations and clinical studies and serial nutritional assays were made of the group. The nutritional assay included measurement of the blood concentration of hemoglobin, plasma carotene, vitamin A and vitamin C, total serum protein, serum albumin and globulin and serum calcium, phosphorus and phosphatase. The clinical examination, in addition to specific tests for tuberculosis, included calculation of per cent standard weight and, in a large number of instances, measurement of bone density.

Twenty-eight persons in the group developed roentgenographic evidence of tuberculosis during the seven years of the investigation. Sixteen of these had active disease and 4 of them died during the study. Twelve had lesions which appeared to be inactive when first detected, but in 4 persons the lesions fluctuated in a visibly favorable way, indicating recent activity.

Nutritional assays of the 28 gave values evenly distributed through the ranges of values for all 1,100 with respect to all blood elements studied except vitamins A and C. All cases of clearly active disease occurred in persons with markedly substandard values for vitamins A and C prior to the development of tuberculosis. An inverse relation thus appeared evident in the development of tubercu-

losis and blood concentration of these vitamins. The degree of probability that such a relation is significant is indicated by statistical computations in the text.

No correlation was evident in the development of tuberculosis and deviation from standard weight or bone density.

Appropriate studies indicated a degree of persistence of nutritional pattern ensuring suitability of the population under investigation for this study.

SUMARIO

Estudio de la Relación de la Nutrición con la Producción de Tuberculosis

Un grupo de 1,100 hombres, sin tuberculosis pulmonar en su primer examen, según demostró el estudio radiológico, fué mantenido en observación durante períodos de tiempo que variaron de un mes a cinco años. El promedio de años de observación por persona fué de 1.5. Un 83 por ciento de los sujetos eran negros y 17 por ciento blancos. Todos tenían de 20 a 45 años de edad. Hiciéronse exámenes radiológicos periódicos y estudios clínicos y valoraciones nutritivas seriadas de los miembros del grupo. Las valoraciones nutritivas comprendieron mediciones de la concentración sanguínea de hemoglobina, carotina plasmática, vitamina A y vitamina C, proteína, albúmina y globulina totales en el suero y calcio, fósforo y fosfatasa en el suero. El examen clínico, además de pruebas específicas para tuberculosis, comprendió cálculos del porcentaje del peso modelo y, en un gran número de casos, mediciones de la densidad ósea.

Durante los siete años de la investigación, 28 sujetos del grupo manifestaron signos roentgenológicos de tuberculosis. Dieciséis de ellos tenían enfermedad activa y 4 de éstos fallecieron durante el estudio. Doce tenían lesiones que parecían inactivas al ser descubiertas, pero en 4 de ellos las lesiones fluctuaban en una forma visiblemente favorable, indicando esto actividad reciente.

Las valoraciones nutricionales de los 28 tuberculosos arrojaron cifras distribuídas uniformemente entre los límites de las cifras correspondientes a todo el grupo de 1,100 con respecto a todos los elementos sanguíneos estudiados, con excepción de las vitaminas A y C. Todos los casos de enfermedad netamente activa ocurrieron en personas con cifras decididamente subnormales de las vitaminas A y C antes de la aparición de la tuberculosis. Púsose, pues, de manifiesto una relación inversa entre la aparición de tuberculosis y la concentración sanguínea de dichas vitaminas. Por medio de cómputos estadísticos en el texto indícase la probable importancia que tiene dicha relación.

No hubo correlación evidente entre la producción de tuberculosis y la desviación de lo normal del peso o de la densidad ósea.

Estudios apropiados indicaron cierto grado de persistencia del patrón nutricional que indicaba que la población en vías de investigación se prestaba para este estudio.

RESUME

Etude de la relation existant entre la nutrition et le développement de la tuberculose

Onze cents hommes, chez lesquels un premier examen radiologique n'avait permis de déceler aucun signe de tuberculose pulmonaire, ont été suivis pendant

des périodes de un à cinq mois. Le nombre moyen représentant les années d'observation par personne est 1, 5. Quatre-vingt trois pour cent des sujets de ce groupe étaient des nègres, dix-sept pour cent étaient des blancs. L'âge de ces hommes s'échelonnait entre 20 et 45 ans. Le groupe a été soumis à des examens radiologiques périodiques, à des études cliniques et à des tests en série de la nutrition. Le test de la nutrition comportaient le dosage de l'hémoglobine dans le sang, du carotène plasmatique, des vitamines A et C, des protéines totales du sérum, de l'albumine, globuline et calcium sériques, du phosphore et des phosphatases. En dehors des tests spécifiques pour la recherche de la tuberculose, l'examen clinique comprenait l'évaluation du pourcentage du poids standard et, très souvent, la détermination de la densité osseuse.

Des signes radiologiques de tuberculose sont apparus chez vingt-huit individus pendant les sept années de l'investigation. Pendant cette période, seize ont présenté des manifestations d'activité de l'affection, quatre d'entre eux ont succombé. Douze avaient des lésions qui semblaient inactives lorsqu'elles ont été découvertes, mais dans quatre de ces cas les lésions se sont modifiées dans un sens favorable, ce qui prouvait leur activité.

Les chiffres obtenus pour les tests de nutrition, effectués chez ces 28 sujets, sont répartis également dans une gamme de valeurs s'appliquant à l'ensemble de ces 1,100 hommes en ce qui concerne tous les éléments du sang étudiés, à l'exception des vitamines A et C. Tous les cas où des manifestations nettement actives de la tuberculose se sont produites étaient ceux dans lesquels la teneur du sang en vitamines A et C était inférieure aux normes avant tout développement du processus tuberculeux. Une relation inverse est donc apparue entre le développement du processus tuberculeux et la concentration de ces vitamines dans le sang. Dans ce texte, le degré de probabilité quant à la valeur de cette relation est indiqué par des statistiques.

Aucune corrélation n'apparaît entre le développement du processus tuberculeux et les déviations observées par rapport au poids-type ou à la densité de l'os.

Des études appropriées ont indiqué un degré de continuité du type de nutrition qui garantissait pour cette étude l'aptitude du groupe soumis à l'investigation.

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BRONCHOGRAPHY IN PULMONARY TUBERCULOSIS[1]

With Histopathologic Correlation in 82 Resections

MAURICE G. BUCKLES, WILLIAM L. POTTS, HORACE B. DAVIDSON, AND WILFORD B. NEPTUNE

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INTRODUCTION

Bronchiectasis, as a complication of pulmonary tuberculosis, considerably modifies the results of treatment directed against the basic lesion. Recognition of this fact has been responsible for several studies designed to define more precisely the exact significance of bronchiectasis in the management of pulmonary tuberculosis.

In the Benjamin Franklin Hospital, all patients suspected of bronchiectasis are studied by bronchography before decision concerning collapse or excision therapy. Consequently, none is treated by major thoracic surgery, e.g., thoracoplasty, decortication, or resection, unless complete bronchographic studies are available preoperatively. Through such practice, better recognition of the entire extent of the disease process is available and more accurate prognosis is possible since it is well recognized that demonstration of bronchiectasis may alter the type and results of the therapy recommended for the patient.

The present report defines the incidence of bronchiectasis in more than 200 patients, as suggested through bronchography. It was possible to correlate these findings with the histopathologic examination in those patients who were subjected to pulmonary resection. An opportunity was also afforded to extend previous observations on a closely related subject, tuberculous bronchitis. An earlier report on tuberculous bronchitis (5) was based on microscopic study of resected pulmonary tissue in 52 of the present series of 82 surgical specimens.

In the previous report, the diagnosis of tuberculous bronchiectasis was based entirely upon discovery of tubercles in walls of ectatic bronchi. More recently, however, but without any change in method of examination, our criteria for differentiating between nonspecific and specific (tuberculous) bronchiectasis have been altered. In an attempt to use diagnostic terminology more precisely, we have further reviewed the relationships of tuberculosis, bronchitis, and bronchiectasis in 222 patients and have reappraised the meaning of descriptive terms employed. The following concept has now evolved.

Parenchymal pulmonary tuberculosis is frequently accompanied by tuberculous infection of bronchi, especially in contiguous areas. Tuberculous infection of bronchi is apt to produce bronchial obstruction, and the latter is an important factor in causation of bronchiectasis. Bronchiectasis is, therefore, often directly due to obstruction, rather than a specific end result of the particular disease causing bronchial lesions. Accordingly, we now make sharp distinction between (a) *tuberculous bronchitis with bronchiectasis* and

[1] From the Benjamin Franklin Hospital, Columbus, Ohio.

(b) *pure tuberculous bronchiectasis*. The former term is applied to dilatation of bronchi accompanying tuberculous bronchitis and presumably due to obstruction resulting from tuberculous bronchitis; while the latter is related to bronchial dilatation resulting from purely locally destructive effects of tuberculosis on bronchial walls. Furthermore, since tuberculous infection of bronchi is so frequently seen in the immediate vicinity of cavities, *tuberculous bronchitis* is employed as a diagnostic term only when significant tuberculous infection of bronchi is observed at a point remote from cavitation.

CLINICAL MATERIAL AND METHOD OF STUDY

Included in this survey were 222 consecutive patients with active pulmonary tuberculosis, in all of whom bronchography and bronchoscopy had been performed. Eighty-one were subsequently subjected to 82 resection procedures; as the result of which, a close correlation is available through surgical specimen examination. The period of study extended from July 1947 to October 1949.

Age, sex, and race: Within this group were 96 men and 126 women. Of the former, 66 were white, 28 Negro, and 2 Asiatic. Among the females, 99 were white and 27 non-white. The age of the men ranged between 14 and 58, with an average of 38 years; that of the women varied from 15 to 63, with an average of 34 years.

TABLE 1

Classification of Pulmonary Tuberculosis in 222 Patients Examined by Bronchography

EXTENT	NUMBER	PER CENT
Minimal	26	11.7
Moderately advanced	74	33.3
Far advanced	122	55.0
Bilateral	107	48.2

Activity status of pulmonary tuberculosis: At the time of bronchography, all of the patients showed roentgenographic evidence of active parenchymal tuberculosis, with positive bacteriologic confirmation.

Volumetric extent of parenchymal tuberculosis: At the time of bronchography, 26 patients (11.7 per cent) were considered to have minimal, 74 patients (33.3 per cent) moderately advanced, and 122 patients (55 per cent) far advanced lesions, as defined by roentgenography and in accordance with National Tuberculosis Association nomenclature (1). This represents a reasonably close sampling of our entire hospital population, but it should be noted that many were subjected to resection after failure of other types of therapy and did not represent pure salvage surgery. The exact extent of parenchymal involvement in members of the series is shown in table 1.

Duration of pulmonary tuberculosis: The known onset of illness was placed in that year in which the patient was first definitely discovered to have pulmonary tuberculosis although bacteriologic examinations may occasionally have been

negative for tubercle bacilli at some time during the ensuing period. The duration of illness, which varied from a few months to many years, is shown in table 2.

Previous collapse therapy: As analyzed subsequently, 125 ipsilateral collapse therapies had previously been instituted and frequently multiple collapse therapy had been used in the same patient.

Technique of bronchography: In the 222 patients studied, iodized oil was introduced transglottically, with visualization of various bronchopulmonary segments effected by proper positioning methods. Other than a few transient mild toxic reactions to the topical anesthetic employed (Pontocaine), no significant early or delayed complication was attributable to bronchography, with the exception of one case of atelectasis to be subsequently described.

Preoperative demonstration of bronchiectasis: Presence or absence of bronchiectasis, as defined by bronchography, is often a matter of individualized interpreta-

TABLE 2

Duration of Pulmonary Tuberculosis in 222 Patients Examined by Bronchography

TIME IN YEARS	NUMBER
0 to 1	83
1 to 2	43
2 to 3	27
3 to 4	7
4 to 5	13
5 to 10	30
10 to 15	15
15 to 20	4

tion, and several competent viewers may express varying ideas on the subject. Accordingly, in the present series, roentgenographic bronchiectasis was diagnosed only when described by a majority of the resident hospital staff and independently agreed upon by the consulting roentgenologist. No attempt will be made to distinguish between saccular, cylindrical, or other forms in the present discussion.

Method of tissue selection in resected specimens: All bronchi were opened in the operating room and immediately examined for presumptive evidence of bronchiectasis, as manifested by bronchial dilatation. At the same time, the presence or absence of obvious bronchitis was established through inspection for ulceration, proliferation, or stenosis. Sections for microscopic study were removed from gross areas of involvement or, in its absence, by random selection of tissue in the large bronchi nearest the hilum. As mentioned previously, bronchial involvement immediately adjacent to cavity was not considered a positive finding.

OBSERVATIONS

Bronchiectasis was suggested by the preoperative findings in 115 of the entire group of 222 patients studied. Among these 115 patients, the condition was

bilateral in 18. In addition, blockage of a common bronchus was shown in 4, and tuberculous tracheostenosis in one (figure 1). In other words, 119 (54 per cent) seemed to reveal either bronchiectasis or tracheobronchial involvement by bronchography. Twenty patients (9 per cent) had bronchoscopic evidence of tuberculous endobronchitis and 15 of this group were women. This compares with the prevalence of bronchiectasis in women noted by others in routine bronchoscopy of tuberculous patients (2, 3, 4).

TABLE 3

Pulmonary Areas Involved with Bronchiectasis as Demonstrated by Bronchography in 115 Patients

PULMONARY AREA (S)	MEN	WOMEN	TOTAL
Right upper lobe	15	28	43
Posterior segment only		1	
Right middle lobe	4	15	19
Right lower lobe	7	19	26
Superior segment only	4	2	
Left upper lobe	28	28	56
Apical posterior segment only	1	1	
Lingular segment only	3	4	
Left lower lobe	16	19	35
Superior segment only	1	3	

TABLE 4

Incidence of Bronchiectasis in Relation to Extent of Pulmonary Involvement

EXTENT OF PULMONARY TUBERCULOSIS	TOTAL EXAMINED	BRONCHIECTASIS		
		Men	Women	Total
Minimal	26	3	8	11 (42%)
Moderately advanced	74	13	22	35 (47%)
Far advanced	122	30	39	69 (57%)

It soon became apparent that bronchial segments implicated in bronchiectasis were those leading into areas of pulmonary pathology (table 3) and that distribution of ectasia is largely dependent upon location of the parenchymal process. Although the frequency of bronchial involvement could be expected to parallel the extent of parenchymal lesions, the correlation was most clear cut with the severe degrees of pulmonary involvement (table 4).

Accuracy of roentgenographic diagnosis of bronchiectasis: The degree of accuracy in roentgenographic diagnosis of bronchiectasis among the 222 patients was determinable only through analysis of findings in those patients who were later subjected to pulmonary resection. This procedure was performed on 81 persons,

among whom 82 resections were carried out on 36 males (44.4 per cent) and 45 females (55.6 per cent), a proportion which reflected the representation of the sexes in the entire group.

In the group of patients who underwent resection, a preoperative diagnosis of bronchiectasis had been made in 60 (73.2 per cent). Of these, 39 (65 per cent) were women and 21 (35 per cent) were men, representing a definite increased prevalence in the female. Pulmonary areas in which bronchiectasis was so suspected by bronchography are shown in table 5 and are similar to findings for the entire group of 222 patients (table 3).

Microscopic postoperative study confirmed *bronchiectasis*, however, in only 44 of the 60 patients in whom it had been diagnosed before surgery. Of this number, nontuberculous bronchiectasis was identified in 42 (43.3 per cent of

TABLE 5

Results of Bronchography in 82 Patients Before Resection Showing Areas Involved in 60 with Roentgenographically Demonstrable Bronchiectasis

PULMONARY AREA	NUMBER
Right upper lobe	23
Posterior segment only	1
Right middle lobe	12
Right lower lobe	13
Superior segment only	6
Left upper lobe	29
Apical posterior segment only	2
Lingular segment only	1
Left lower lobe	18
Superior segment only	3

the operated group), while in only 2 instances was the lesion considered truly tuberculous in character. Chronic nonspecific *bronchitis* was apparent in specimens from 17 patients (17.5 per cent), while true tuberculous bronchitis was identifiable in 35 (36.1 per cent). This differentiation has been further subdivided according to sex and extent of parenchymal involvement and is shown in table 6.

Further analysis revealed that tuberculous bronchitis, without bronchiectasis, occurred in 14, a typical illustration of which is seen in figure 2. Bronchiectasis, without tuberculous bronchitis, was found in 21, an example of which is shown in figure 3. Bronchiectasis and endobronchial tuberculosis were coexistent in 21, representative of which is the bronchogram reproduced in figure 4. As stated previously, in only 2 cases could the diagnosis of pure tuberculous bronchiectasis be confirmed according to the criteria already outlined. A summary of these findings is shown in table 7.

In the resected specimens, the predominant pulmonary lesions were chronic ulcerative in 48 instances; in these lesions the gross diameter of the cavities varied between 0.5 and 11 cm. Fibrocaseous tuberculosis was present in 28 specimens (table 6). In 3 specimens, the pulmonary tuberculosis was minimal in extent, while in 25 it was moderately advanced and in 53 far advanced. The segments of pulmonary tissue actually removed are identified in table 8.

It is clear that suspected preoperative, and proved postoperative, bronchiectasis did not parallel one another in frequency. The presence of bronchiectasis

TABLE 6

Pathologic Diagnoses Based on Examination of 82 Resected Specimens

DIAGNOSIS	EXTENT OF PULMONARY TUBERCULOSIS						Total
	Men			Women			
	Minimal	Moderately advanced	Far advanced	Minimal	Moderately advanced	Far advanced	
Chronic ulcerative pulmonary tuberculosis		7	20		2	19	48
Fibrocaseous pulmonary tuberculosis	1	2	3	1	11	10	28
Chronic bronchitis (nonspecific)		3	5		4	5	17
Bronchiectasis (nonspecific)		6	9	1	6	20	42
Tuberculous bronchitis		4	12	2	4	13	35
Tuberculous bronchiectasis					1	1	2
No tuberculosis							1

TABLE 7

Type of Bronchial Disease in 82 Resected Specimens

PATHOLOGY	NUMBER	PER CENT
Tuberculous bronchitis without bronchiectasis	14	17
Bronchiectasis without tuberculous bronchitis	21	26
Bronchiectasis with endobronchial tuberculosis	21	26
Tuberculous bronchiectasis	2	2

was diagnosed roentgenographically in 60 cases but confirmed through microscopic study of the tissues in only 44. Several possible explanations for this discrepancy occur and include: (a) "over-reading" of bronchograms; (b) presence of minimal ectatic changes (possible so-called pseudobronchiectasis) overlooked on gross examination of the resected specimen (figure 5); (c) misinterpreting bronchial distortion secondary to tuberculous bronchitis as minimal bronchiectasis (figure 2); and (d) confusion of contrast medium in cavities with bronchiectasis (figure 6).

It was also evident among the 9 female patients with a preoperative bronchoscopic diagnosis of tuberculous bronchitis that post-surgical confirmation was

obtainable in only 3 patients. Although criteria for endoscopic diagnosis were rigid, including definite ulceration, proliferation, or stenosis, the discrepancy may have occurred as a consequence of resection of a bronchus distal to an area of disease visualized through the bronchoscope. This seems to confirm findings reported by Meissner and associates (7).

It was also concluded that antecedent ipsilateral collapse procedures, applied to that lung in which bronchiectasis was apparently demonstrated, did not

TABLE 8

Pulmonary Tissue Resected in 82 Operations

OPERATION	NUMBER	
Pneumonectomies		24
Right	8	
Left	16	
Lobectomies and Segmental Resections		58
Right upper lobe	16	
Right upper lobe and right middle lobe	4	
Right upper lobe and right lower lobe	1	
Right upper lobe and right middle lobe and superior division right lower lobe	2	
Right upper lobe and superior division right lower lobe	3	
Apical division right upper lobe and right middle lobe and right lower lobe	1	
Posterior division right upper lobe and right middle lobe and right lower lobe	1	
Right middle lobe and right lower lobe	2	
Lateral division right lower lobe and right lower lobe	1	
Left upper lobe	14	
Lingula left upper lobe	1	
Left upper lobe and superior division left lower lobe	2	
Apical division left upper lobe	5	
Apical posterior left upper lobe and superior division left lower lobe	1	
Left lower lobe	4	
Total		82

appear to have contributed significantly to development of this condition. Reference to table 9 will indicate the basis for this observation in which pneumothorax and pneumoperitoneum were excluded from calculations unless maintained for at least three months.

No significant complications to bronchography occurred in the entire series with exception of the following instance of atelectasis.

Case Report

M. L., a 23-year-old non-white female with far advanced lesions of seven years' duration, had previously received bilateral pneumothorax. Because pneumothorax therapy was unsuccessful, a left-sided seven-rib thoracoplasty was

subsequently performed without preliminary iodized oil studies. Four years later, the patient's tuberculosis relapsed with the appearance of cough, hemoptysis, and sputum positive for tubercle bacilli, and she was re-admitted to the hospital. Bronchography revealed extensive subthoracoplasty bronchiectasis (figure 3). This did not impress the writers as true thoracoplasty failure in the strict sense; it could possibly have represented bronchiectasis coexisting at time of thoracoplasty or subsequent development of such a condition.

Following bronchography, postural drainage was employed. Approximately four hours later, however, the patient developed dyspnea, anterior thoracic pain, and fever, and roentgenograms confirmed a clinical impression of atelectasis. Tracheal aspiration failed to relieve the symptoms, and bronchoscopy was performed. A considerable amount of retained iodized oil and bloody sputum was aspirated, with complete relief of complaints and almost immediate re-aeration as demonstrated by chest films.

TABLE 9

Previous Therapy for the Lung Examined by Bronchography

THERAPY	DEMONSTRABLE BRONCHIECTASIS	BRONCHOGRAM CONSIDERED NORMAL
None	41	—
Phrenic interruption alone	21	16
Phrenic interruption with pneumoperitoneum	23	27
Pneumothorax	62	66
Oleothorax	3	1
Cavernostomy	1	2
Thoracoplasty	15	11

COMMENT

Bronchiectasis was demonstrated bronchographically in 115 of a series of 222 consecutively examined patients, and 82 of the 115 subsequently underwent pulmonary resection. In the latter group, however, the preoperative diagnosis of bronchiectasis was confirmed by study of the resected tissues in only 53.7 per cent. Moreover, in only 2.44 per cent of the entire operative group was the bronchiectasis found to be tuberculous according to criteria set forth in the present report. The diagnosis of tuberculous bronchiectasis, therefore, is apparently sometimes made without complete appreciation of proper definition and pathology.

It is suggested that bronchiectasis usually follows tuberculous bronchitis or may occasionally be the end result of extrinsic traction on bronchial walls by parenchymal scar tissue. The present studies indicate that the frequency of the underlying bronchitis is related to the extent of the parenchymal tuberculosis although the ratio is not a direct one. This finding is in contrast to the findings of others who also find tuberculous involvement of bronchi in at least 50 per cent of autopsied cases but believe that the frequency of the condition bears a direct relation to extent of pulmonary involvement (3).

In the present study, bronchitis was distributed evenly throughout the entire

bronchopulmonary tree and was dependent only upon anatomical location of the responsible parenchymal lesion; this is not in agreement with Fujikawa and Ackerman (8) who, on the basis of 34 pulmonary resections, reported that tuberculous endobronchitis was uncommon in upper lobe tuberculosis and was most frequently found as a complication of lower lobe bronchiectasis.

It was possible in the present study to identify the significant microscopic lesion of tuberculous bronchitis postoperatively nearly four times as frequently as had been suspected on the basis of pre-surgical bronchoscopy. No doubt the bronchial involvement results from direct implantation of tubercle bacilli on bronchial mucosa; this, in turn, subsequently produces broncho-obstruction and eventually bronchiectasis. The writers believe that previous ispilateral collapse therapy is not a significant factor in the production of such bronchial dilatation.

Although the writers believe that bronchiectasis in the tuberculous is most often actually nontuberculous, it is believed that bronchography is indicated, along with bronchoscopy, in all cases under consideration for major thoracic surgical procedures. As a result, the complete extent of pathologic involvement may be more accurately predicted and a better estimation of prognosis becomes available because demonstration of bronchiectasis in cases otherwise treatable by thoracoplasty strongly suggests that resection may be necessary to bring about sputum "conversion." Moreover, in patients selected for resection on other

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FIG. 1. G. M., 62-year-old white female with moderately advanced pulmonary tuberculosis of five years' duration. Bronchogram demonstrated partial stenosis of trachea and right common bronchus, with complete blockage of left common bronchus. Bronchoscopy revealed severe ulcerostenotic type of tuberculous bronchitis.

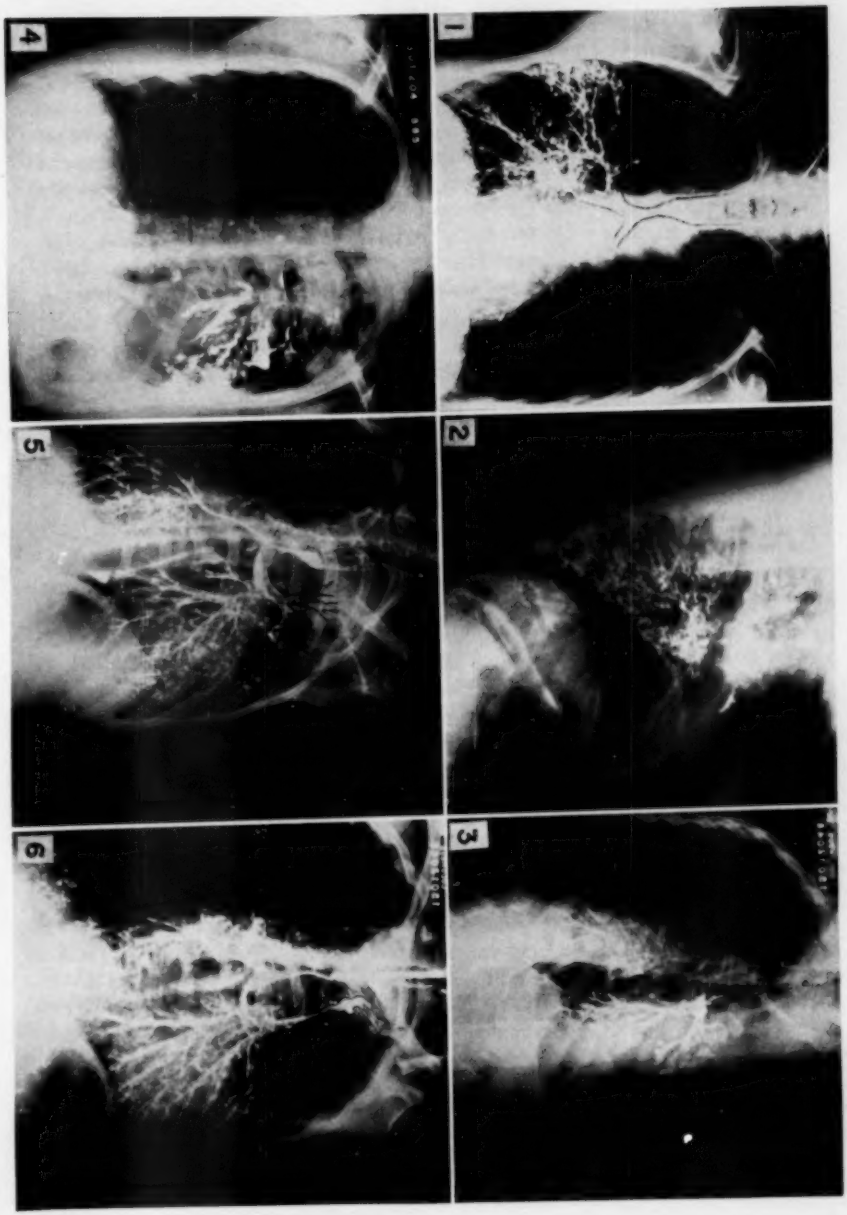
FIG. 2. J. W., 40-year-old white male with far advanced pulmonary tuberculosis of three years' duration and severe diabetes mellitus. Previously underwent resection of right middle lobe for nonspecific bronchiectasis. Bronchogram (right lateral view) interpreted as demonstrating bronchiectasis of right upper lobe. Treated by pneumonectomy. Surgical specimen revealed chronic ulcerative tuberculosis, extensive tuberculous bronchitis, but no bronchiectasis.

FIG. 3. M. L., 23-year-old non-white female. Demonstration of bronchiectasis of left lung, underlying previous thoracoplasty. Treated by pneumonectomy. Surgical specimen revealed chronic fibrocaseous tuberculosis, chronic nonspecific bronchitis, and bronchiectasis. No evidence of endobronchial tuberculosis.

FIG. 4. G. G., 28-year-old non-white female with far advanced pulmonary tuberculosis of five years' duration. Previously treated by left pneumothorax. Bronchogram demonstrates extensive bronchiectasis of left lung, with contrast filling of several tuberculous cavities. Treated by pneumonectomy. Surgical specimen revealed chronic ulcerative tuberculosis, tuberculous bronchitis, and bronchiectasis.

FIG. 5. W. P., 29-year-old white female with far advanced pulmonary tuberculosis of one year's duration. Bronchogram seems to demonstrate bronchiectasis of left upper lobe. Treated by resection of left upper lobe. Surgical specimen revealed chronic ulcerative tuberculosis and true tuberculous bronchiectasis.

FIG. 6. L. D., 31-year-old non-white male with pulmonary tuberculosis of ten years' duration; cavity was never demonstrated preoperatively and parenchymal lesion was interpreted as minimal in extent. Bronchogram interpreted as demonstrating bronchiectasis of the apical posterior segment of left upper lobe. Treated by segmental resection. Surgical specimen revealed tuberculous cavity without evidence of bronchiectasis or tuberculous bronchitis.



FIGS. 1–6

grounds, e.g., giant cavity, lower lobe excavation, atelectasis, thoracoplasty failure, or bronchostenosis, knowledge of the presence of bronchiectasis exerts an influence in deciding the amount of pulmonary tissue to be removed.

In the presence of definite bronchiectasis and "positive sputum" but without obvious parenchymal lesion, the present studies suggest that a complicating tuberculous bronchitis is usually responsible. In the past many such patients have been casually labeled as having tuberculous bronchiectasis. The presence of tuberculous bronchitis probably explains occasional cases encountered in which the sputum contains tubercle bacilli, the chest film is normal, and there is no evidence of bronchial dilatation after instillation of iodized oil. Such instances probably represent cases of tuberculous bronchitis beyond the range of bronchoscopic vision.

The high incidence of tuberculous bronchitis in the present series may well explain the failure of previous collapse procedures employed in certain cases.

SUMMARY

The results of bronchographic study in 222 tuberculous patients have been reviewed, and in 82 patients the preoperative findings were compared with the results of examination of resected specimens.

Bronchiectasis was demonstrated through the use of iodized oil in 115 (51.8 per cent) of the 222 cases. Among the patients eventually subjected to resection, bronchiectasis was diagnosed roentgenographically in 60 (73.2 per cent) but was confirmed by postoperative microscopic study in only 44 (53.7 per cent).

The discrepancy in the results obtained by the two methods of examination may be explained by "over-reading" of bronchograms, overlooking of borderline cases during pathologic examination, or by confusing iodized oil-filled cavities with bronchiectatic dilatations. The principal cause, however, would seem to be misinterpretation of distortions due to tuberculous bronchitis.

Only 20 (9 per cent) of the resection group showed bronchoscopic evidence of tuberculous endobronchitis, according to the writers' criteria. Nevertheless, postoperative microscopic study revealed the presence of tuberculous endobronchitis in approximately four times this number. The writers believe this entity is often responsible for the discharge of *M. tuberculosis* in the sputum of patients with so-called tuberculous bronchiectasis since true tuberculous bronchiectasis was found in less than 3 per cent of all bronchial specimens studied after surgical removal.

The final microscopic diagnoses in the resection group included: (a) chronic nonspecific bronchitis in 17 specimens, (b) tuberculous bronchitis in 35, (c) nonspecific bronchiectasis in 42, and (d) true tuberculous bronchiectasis in only 2 specimens.

It is suggested that the term *tuberculous bronchitis* be employed only when significant tuberculous endobronchial involvement is demonstrated at a point distant from a satellite cavity; that *tuberculous bronchiectasis* be diagnosed only through evidence of extensive destruction of bronchial walls by tuberculosis; and that *tuberculous bronchitis with bronchiectasis* be used to describe

the bronchial dilatation which may accompany bronchial tuberculosis but is not a direct result of the locally destructive effects of tuberculosis.

In no instance was bronchiectasis detected on microscopic examination when it had not been shown on preoperative bronchography. Although many sections were obtained in the cases of the present study, it is believed that a single section of a bronchus is sufficient to make reasonably certain that bronchiectasis in that region is nontuberculous.

SUMARIO

La Broncografía en la Tuberculosis Pulmonar: Con la Correlación Histopatológica en 82 Resecciones

Repasados los resultados del estudio broncográfico de 222 tuberculosos, preséntase además la correlación con 82 ejemplares resecados.

Se descubrió bronquiectasia por medio del empleo de aceite yodado en 115 (51.8 por ciento) de los 222 casos. Entre los enfermos resecados más tarde, fué diagnosticada del mismo modo en 60 (73.2 por ciento), pero sólo la confirmó el estudio microscópico postoperatorio en 44 (53.7 por ciento).

Cabe explicar esa discrepancia por la interpretación exagerada de los broncogramas, el pasar por alto casos limítrofes durante el examen anatomopatológico, o tomar por dilataciones bronquiectáticas cavernas llenas de aceite yodado. No obstante, parece que la causa principal consiste en interpretación errónea de deformaciones debidas a la bronquitis tuberculosa.

Sólo 20 (9 por ciento) del grupo resecado revelaron, conforme a las pautas del A., signos broncoscópicos de endobronquitis tuberculosa; y sin embargo, el estudio microscópico postoperatorio reveló su presencia aproximadamente en el cuádruple de ese número. Opinan los AA. que dicha entidad constituye a menudo la causa de la bacteriología positiva en la llamada bronquiectasia tuberculosa, pues se descubrió verdadera bronquiectasia tuberculosa en menos de 3 por ciento de todos los bronquios estudiados después de haberlos extirpado quirúrgicamente.

Los diagnósticos microscópicos definitivos en el grupo resecado comprendieron: (a) bronquitis anespecífica crónica en 17 ejemplares; (b) bronquitis tuberculosa en 35; (c) bronquiectasia anespecífica en 42; y (d) verdadera bronquiectasia tuberculosa solamente en 2.

Propónese que no se emplee el término de *"bronquitis tuberculosa"* más que cuando se decubra significativa invasión endobronquial tuberculosa en un punto alejado de una caverna satélite; que sólo se diagnostique *"bronquiectasia tuberculosa"* a base de signos de extensa destrucción de las paredes bronquiales por la tuberculosis; y que se use *"bronquitis tuberculosa con bronquiectasia"* para describir la dilatación bronquial que acompaña a la tuberculosis bronquial, pero no se debe a los efectos destructores locales de la última.

En ningún caso se descubrió microscópicamente bronquiectasia sino la había revelado la broncografía preoperatoria.

Parece, según también indican previos estudios de los AA., que un solo corte de un bronquio basta para sentirse razonablemente seguro de que no es tubercu-

losa la bronquiectasia de la región dada; aunque en estos casos se tomaron muchos cortes, opínase que un corte a través de una zona sospechosa resulta adecuado.

RESUME

(*Bronchographie dans la tuberculose pulmonaire: Corrélation histopathologique dans 82 résections*)

Les résultats de l'étude de bronchographies de 222 tuberculeux ont été examinées et 82 résections ont été présentées.

La présence de la bronchiectasie a été démontrée dans 115 cas sur 222 (51, 8%) à l'aide d'huile iodée. Parmi les patients devant subir une résection, la bronchiectasie a été diagnostiquée dans 60 cas (73, 2%) mais elle ne fut confirmée par l'examen histologique postopératoire que dans 44 cas (53, 7%).

Cette variation peut s'expliquer par une interprétation erronée des bronchogrammes, par une interprétation histopathologique erronée des cas-limite ou par une confusion des champs pulmonaires remplis de lipiodol avec la dilatation bronchique. Cependant, la principale cause d'erreur semble être due à la présence de la tuberculose des bronches.

Seulement 20 (9%) des opérés montraient des signes de tuberculose endobronchique en accord avec nos normes. Toutefois, les études histologiques postopératoires montrent la présence de BK dans 4 fois plus de cas. Les auteurs croient que cette entité est souvent responsable des résultats bactériologiques positifs dans la bronchiectasie pseudo-tuberculeuse. Depuis, dans les études faites sur les bronches après ablation chirurgicale, nous n'avons observé de vraies bronchites tuberculeuses que dans moins de 3% des cas.

Le diagnostic histologique final dans les groupes réséqués montrent: a) 17 spécimens de bronchite chronique non spécifique; b) 37 tuberculoses des bronches; c) 42 bronchiectasies non spécifiques; d) seulement 2 bronchiectasies vraiment tuberculeuses.

Les auteurs pensent que le terme de "*bronchite tuberculeuse*" ne peut être utilisé que lorsque l'atteinte de tuberculose endobronchique siege en un point distant d'une cavité satellite; que la "*bronchiectasie tuberculeuse*" ne soit diagnostiquée que lorsqu'il existe une destruction de la paroi bronchique par le BK; et que "*bronchite tuberculeuse avec bronchiectasie*" ne soit employé que pour décrire une dilatation bronchique associée à la tuberculose des bronches, mais qui n'est pas la conséquence d'une action locale destructrice.

En aucun cas la bronchiectasie n'a été discutée ici du point de vue histologique quand elle n'a pas été observée sur les bronchographies préopératoires.

Les auteurs croient que, comme dans leurs études précédentes, une simple section de bronche est suffisante pour s'assurer de l'étiologie non tuberculeuse de la bronchiectasie en ce point. Bien que de nombreuses sections aient été faites dans ces cas, les auteurs pensent qu'il suffit d'une seule section à travers un territoire suspect.

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TUBERCULOUS MENINGITIS: CORRELATION OF THERAPEUTIC RESULTS WITH THE PATHOGENESIS AND PATHOLOGIC CHANGES[1, 2, 3]

I. General Considerations and Pathogenesis

OSCAR AUERBACH[4]

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INTRODUCTION

Since the introduction of streptomycin as an antimicrobial agent in the treatment of tuberculosis, many reports of dramatic results in the treatment of tuberculous meningitis have appeared in the medical literature. This therapy has been more successful in children than in adults. Cures have been reported, and in fatal cases the course of illness has been prolonged from three weeks to many months and in many instances to one or more years.

The present study has been undertaken in order to explain differences in the mode of development of tuberculous meningitis in children and in adults and to determine whether this might influence the course of treatment.

OBSERVATIONS

From 1932 to 1947, 2,333 autopsies were performed on patients who died of some form of tuberculosis at Sea View Hospital. Of these patients, 108 (4.6 per cent) succumbed to tuberculous meningitis.

Composition of Group

Age: The youngest patient in the group was seven months of age, while the oldest was sixty-seven years of age. For purposes of classification, the upper limit of childhood is placed at twelve years of age. Forty-one patients died during this period of life. These patients represent 42.2 per cent of the 97 childhood fatalities in this series due to tuberculosis. Sixty-seven deaths due to meningitis occurred in patients who ranged in age from thirteen to sixty-seven years. These deaths represented 2.9 per cent of the 2,236 deaths due to tuberculosis in this age group. Only 5 of these cases occurred between the ages of thirteen and nineteen years. The peak incidence in the adolescent and adult group was in the fourth decade, with great decrease in the subsequent decades. Only 3 cases were observed in the seventh decade.

Sex and color: Of the 108 patients, 74 (68.5 per cent) were males and 34 (31.5

[1] From the Laboratory Service, Halloran Veterans Administration Hospital, Staten Island 2, New York.

[2] Sponsored by the Veterans Administration and published with approval of Chief Medical Director. The statements and conclusions published by the author are a result of his own study and do not necessarily reflect opinion or policy of the Veterans Administration.

[3] This study was made at Sea View Hospital while the author was pathologist at that institution.

[4] Home address: 121 Hamden Avenue, Staten Island 6, New York.

per cent) were females. This distribution almost paralleled the sex distribution in the general autopsy cases.

There were 64 (59.2 per cent) Negroes and 44 (40.8 per cent) white patients in this series. In the entire autopsy group during this same period only 37.6 per cent of the patients were Negroes.

Extrameningeal Tuberculosis

Since tuberculous meningitis is always secondary to an extrameningeal lesion, scrutiny of the disease as it affects other structures is of importance in any evaluation of this facet of the disease.

Technique of examination of the lungs: The lungs and tracheobronchial tree are removed intact. The lungs are filled with Kaiserling I or formalin through the trachea by means of a funnel and attached rubber tube. If any tears are present in the pleura, they are tied with cotton sutures so that no fluid escapes from the lungs. When the lungs are filled with the fluid, the trachea is firmly tied near the cut end and the specimen is placed in a large container of formalin in a position similar to that it had in the body. After twenty-four hours of fixation, coronal sections of the lungs are made with a long knife. These sections are made along the course of the bronchi. The tracheobronchial lymph nodes are cut in a similar fashion.

TABLE 1

Age Distribution

NUMBER OF CASES	AGE
10	1–12 months
28	13 months–9 years
8	10–19 years
19	20–29 years
25	30–39 years
10	40–49 years
5	50–59 years
3	More than 60 years

Pulmonary lesions (children): A progressive primary complex was present in 12 of 41 children. In each case the lungs were studded with small and large caseous foci which were part of an extensive hematogenous dissemination. In 4 other children, the primary focus had undergone liquefaction with the formation of a cavity containing calcified particles. These 4 cases were considered to be instances in which the progressive primary complex had reversed its course toward anatomic healing.

In the remaining 25 children the primary complex was either in an advanced stage of healing, i.e., granulation tissue was still present in portions of the capsule, or it was completely healed, the capsule being composed only of hyalinized connective tissue. Four of these children ranged from two to seven years of age and showed no other evidence of tuberculosis. Seventeen of the remaining 21 patients had scattered miliary foci within the lungs. These foci were observed at

different stages of anatomic healing, but in any one case they were all at the same stage of development. In some instances a giant cell lying in the zone of connective tissue furnished the only evidence of the tuberculous origin of the lesions. Occasionally, the foci were first discovered on microscopic examination. Similar foci showing the same degree of healing were frequently present in the spleen but were noted less frequently in the liver. In 4 other children the healed primary complex was associated with an acute miliary tuberculosis of the lungs. Their ages varied from seven to twelve years, and each of these patients had some form of skeletal tuberculosis. The miliary foci were fresh lesions unrelated to the primary complex, being part of a generalized hematogenous dissemination secondary to the skeletal tuberculosis.

Pulmonary lesions (adolescents): Only 5 persons were in the age group between thirteen years and nineteen years of age. In 3 patients (13, 14, and 15 years old) the lungs contained a healing primary focus and occasional miliary foci. The other 2 (16 and 19 years of age) showed an acute generalized miliary tuberculosis. In the remainder of this paper these 5 cases are considered as part of the adult group.

Pulmonary lesions (adults): There were 34 patients with chronic pulmonary tuberculosis. Twenty-two of these had miliary tubercles in the lower portions of the lungs, which were associated with a generalized miliary tuberculosis. It has previously been shown that this type of dissemination is secondary to an active extrapulmonary tuberculous process (1).

Acute miliary tuberculosis was the only pulmonary disease in 26 patients. These cases, taken with the 22 cases accompanying chronic pulmonary tuberculosis, bring the total number of cases of acute generalized miliary tuberculosis to 48. The miliary foci in each of the cases were numerous and were at different stages of anatomic development, indicating that there had been multiple seedings. In a few instances, however, only a few foci were encountered. In some cases these were found only in the lungs. In others, they were noted in both the lungs and abdominal viscera (liver and spleen).

In only 2 of the 62 adults were the lungs free of tuberculous changes other than a healed primary complex. In both cases an active extrapulmonary tuberculosis was present. Unfortunately, serial sectioning of the brain was performed in only one of the 2 cases and here many foci (tuberculomas) were found in the brain parenchyma, one of which was contiguous with the meninges. No foci were present in the other viscera. In the second case occasional miliary foci in an advanced state of healing were found in the spleen and liver.

Skeletal and Urogenital Tuberculosis

From table 2 it may be seen that in 55 (51 per cent) of the 108 cases evidence of active tuberculosis was found in the urogenital and/or skeletal systems; of these 55 patients, only 5 were children. The genital and skeletal systems were involved in equal proportions, while renal tuberculosis occurred much less frequently. A close relationship obtained between isolated extrapulmonary tuberculosis and acute generalized miliary tuberculosis. Forty-four (88 per cent) of the adults showed an acute hematogenous dissemination.

In 2 instances, the involvement of the meninges occurred as a result of direct extension of the disease from a neighboring structure. In one case the meningitis was derived from a tuberculous osteomyelitis at the base of the skull. The dura was the source of leptomeningeal disease in the other case, and the underlying bone was free of disease. Both patients had an active genital tuberculosis, and one had chronic pulmonary tuberculosis.

TABLE 2

Skeletal and Urogenital Tuberculosis

RENAL	GENITAL	GENITAL SKELETAL	SKELETAL	RENAL GENITAL	SKELETAL RENAL GENITAL
1	17	12	18	4	3

DISCUSSION

In the present study, a marked difference in the incidence of meningitis in fatal cases of tuberculosis among children (42.2 per cent) and in adults (2.9 per cent) has been observed. A similar distribution has also been noted by Steinmeier (2). Tuberculous meningitis in children is most often related to a spread from a primary complex. The close relationship between the primary complex and tuberculous meningitis is substantiated by the ages in which the two conditions are most frequent. The greatest number of both occurred between the ages of one and three years. A survey of the literature emphasizes the fact that the peak incidence of tuberculous meningitis occurs between one and three years and that it decreases each year thereafter. Orosz (3), in a review of the world literature of tuberculous meningitis in children, found that approximately one-fifth of the cases occur in the second year and that approximately four-fifths occur within the first six years of life. The question of how early in life tuberculous meningitis may occur has been reviewed by Seigel (4). He does not accept the two cases of Bosselut and Herben and Asserson (5, 6) as having occurred before two months of age because they were taken from clinical diagnoses and not substantiated by autopsy findings. The earliest case in his series occurred in the third month. He states that it is rare in the third month and frequent thereafter.

The large number of Negroes in the present series of patients, 64 (59.2 per cent), is quite significant since they compose only 37.6 per cent of the total number of patients who died from all forms of tuberculosis. This disproportion is attributed to two factors: (*1*) The progressive primary complex is most frequently seen in Negroes. This form of tuberculosis is prone to extensive hematogenous disseminations which may serve as a source of tuberculous meningitis. (*2*) Skeletal tuberculosis occurs with particular frequency in the Negro population and is frequently associated with meningitis. A study of the general autopsy series shows that there is a greater tendency for Negroes to develop a larger primary complex and thus a greater tendency toward hematogenous dissemination. It is this dissemination which also leads to the frequent occurrence of skeletal tuberculosis in this race. Skeletal tuberculosis with its subsequent

miliary dissemination is therefore an important factor in the development of meningitis.

Evidence of miliary seeding in the different organs was present in 53 (79 per cent) of the 67 adults in the present series. This appears to corroborate Huebschmann's (7) statement that in adults meningitis tends to accompany generalized miliary tuberculosis and that the meningeal disease occurs weeks after the onset of the hemic disease.

The frequent occurrence of miliary tuberculosis and tuberculous meningitis has been noted by a number of authors and has been variously interpreted. Many of the earlier writers on this subject emphasized the high incidence of both these forms of tuberculosis in the spring (8, 9, 10, 11). Some believed the tuberculous meningitis to be a special form of miliary tuberculosis (8, 9).

It is the writer's opinion that, with the exception of cases in which a tuberculous focus in the skull or dura extends to the meninges, tuberculous meningitis is related to and represents the result of an acute hematogenous dissemination of the infection. The blood-stream invasion in children usually arises from the primary complex, while in adults it usually is secondary to an active extrapulmonary tuberculous process.

Hematogenous dissemination from a primary complex occurs while the latter is still at an early stage of development. Once encapsulation of the primary focus occurs, invasion by tubercle bacilli of the blood stream via the lymphatics is halted at the source. As a result of this early dissemination, tuberculous foci develop in the various organs in the body. These are found most frequently in the abdominal viscera (e.g., liver, spleen, kidneys) and the lungs, but are noted less frequently in the brain and other organs. The number and size of the foci depend upon the extent of the primary infection. In most instances the tubercles in the different structures undergo healing. The smaller foci are converted into small scars which give no clue to their origin. Larger foci, which often have a caseous center, may also be replaced by scar tissue or may remain as an encapsulated caseous focus in which calcium salts are later deposited. Such foci are most frequently found in the liver and spleen and occasionally are encountered in the brain.

The primary complex and its resulting hematogenous foci usually show a natural healing tendency which produces no untoward effects. On the other hand, tuberculous meningitis generally develops while the primary complex and the hematogenous foci in the different organs are in an advanced stage of healing. Occasionally, healing of the hematogenous foci may obliterate the characteristics which would mark them as tuberculous, and calcified foci in the spleen and liver may furnish the only evidence of dissemination.

Since the primary complex usually develops in early life, cases of meningitis derived from it appear in younger children. The meningitis in these cases is often associated with a generalized miliary dissemination or, occasionally, with a large nodular tuberculosis when associated with a progressive primary complex. Cases of meningitis associated with advanced healing of the primary complex and complete or almost complete healing of the miliary dissemination generally

occur in older children. Some authors (12, 13) have noted that miliary tuberculosis and tuberculous meningitis are more frequently found in younger than in older children. The observations of Koch (11) in 1912 are of interest in this respect. In a study of a large series of cases of tuberculous meningitis he found only two cases in which there was calcification of foci in the tracheobronchial lymph nodes. In the remaining cases, foci with signs of an active process were still present. He concluded that tuberculous meningitis no longer occurs when the primary focus is chalky.

Lincoln (14) emphasized the fact that miliary tuberculosis was many times more frequent in children dying of meningitis while the primary lesion was fresh than after the focus had shown evidence of calcification.

Thus, with the exception of the few instances in which an active extrapulmonary tuberculosis exists, the development of tuberculous meningitis in children is secondary to a hematogenous dissemination from the primary complex. Once the primary complex heals, the source of the seeding is walled off and the opportunity for meningeal infection is lost.

Conditions are different in the adult. It has been shown that in adults an acute generalized miliary tuberculosis is secondary to, and the result of, an active extrapulmonary tuberculosis (1).

The frequent association of active extrapulmonary tuberculosis and tuberculous meningitis in adults has been noted in previous studies of this disease (15, 16, 17, 18). The extrapulmonary lesions are most often in the skeletal and urogenital systems but may also involve the serous surfaces. Progressive involvement of these structures is almost invariably characterized by caseous tuberculosis of the regional lymph nodes. Unlike the primary complex, the caseous involvement of the regional lymph nodes does not occur until late in the course of the disease. The tubercle bacilli are carried by way of the lymphatics to the venous circulation. A generalized blood-stream dissemination takes place with the resultant development of miliary foci in the various organs.

In common with others who have studied the relationship between generalized miliary tuberculosis and tuberculous meningitis, the present writer has found that, when both conditions coexist, the meningitis may develop after the appearance of the first dissemination. Simultaneously, there is evidence of multiple hematogenous disseminations in the form of miliary foci at different stages of anatomic healing. The multiple seedings are derived from the still active extrapulmonary tuberculous process.

The discrepancy between the time that a miliary tuberculosis is first observed and the time that a tuberculous meningitis develops has been the subject of much discussion. Korteweg (19) believes that the tubercle bacilli are deposited in the leptomeninges with the formation of tubercles. Tuberculous meningitis develops following dissemination of tubercle bacilli from these foci through the arachnoid fluid. It is his opinion that the more tubercles present, the greater is the likelihood of this secondary infection of the meninges.

It is of interest that in the present series there were only 34 cases of chronic pulmonary tuberculosis in which tuberculous meningitis developed and, in 22 of

these, generalized miliary tuberculosis secondary to an active extrapulmonary tuberculosis preceded the meningitis. Two conclusions may be drawn from this, namely: (*1*) that tuberculous meningitis occurring in the presence of chronic pulmonary tuberculosis is an infrequent occurrence; and (*2*) that in most instances where both occur there is an associated extrapulmonary and generalized miliary tuberculosis. Similar observations were made by Engel (12) and Cameron (20). The former stated that the occurrence of a true phthisis and tuberculous meningitis is most infrequent.

Until the studies of Rich and McCordock (21, 22), it had been generally considered that most cases of tuberculous meningitis were the result of a direct infection by way of the blood stream during an extensive dissemination of tubercle bacilli throughout the body.

Hirschberg (23) in 1887, however, stated that the usual form of tuberculous meningitis has its origin in a tuberculous focus in the brain. He claimed that, in addition to the tuberculous meningitis, there was an acute generalized miliary tuberculosis together with the simultaneous occurrence of isolated foci in the brain and that there could be no other source of infection of the leptomeninges but the focus in the brain. Askanazy (24) in 1910 had raised the question of whether the hematogenous infection has anything to do with the tuberculous meningitis. He pointed out that the experiments of Cornil and Bezancon show that the injection of tubercle bacilli into the carotid arteries of dogs and rabbits did not lead to infection of the meninges or brain but that Sicard (25) produced meningeal tuberculosis in two of six rabbits by injecting the carotid artery and tying off both jugular veins and the injected artery.

The studies of Biber in 1911 (26) are of interest in this connection. He carefully sectioned the brain in seventeen cases of tuberculous meningitis and found tuberculous foci in thirteen. In eleven of the brains a parenchymal lesion was continuous with the inflammatory process in the leptomeninges, and he interpreted this as an extension of the tuberculous process from the leptomeninges into the brain substance.

Rich and McCordock (22) found a number of objections to the universally accepted views, namely:

> (*1*) That some cases of advanced miliary tuberculosis show no meningitis; (*2*) that there are cases of meningitis without miliary tuberculosis; (*3*) that, when meningitis and generalized miliary tuberculosis do occur together, there is a lack of correlation between the age and character of the meningeal lesions and the miliary tubercles in other viscera. (*4*) In contrast to the failure of meningitis to develop in the experimental animal as a direct result of even massive blood-stream infection, infection can be produced by the injection of tubercle bacilli into the subarachnoid space. Following the injection of bacilli into the blood stream, if a focal tubercle develops adjacent to the leptomeninges, it may undergo caseation and discharge tubercle bacilli into the subarachnoid space.

Rich and McCordock concluded that tuberculous meningitis ordinarily results from the discharge of bacilli directly into the meninges from a local caseous focus. The site of this focus may by a tuberculous focus (tuberculoma) within the brain, a caseous meningeal plaque, or a focus within the skull. They stressed

the difficulty of finding the caseous foci which cause the meningitis. The frequently small size of these foci necessitates cutting the brain into sections not more than 3 mm. in thickness. Rich and McCordock stress the fact that a caseous meningeal plaque often may be the origin of tuberculous meningitis (it occurred in approximately one-fourth of their cases). They emphasized that all these foci must be studied carefully and that it is only by microscopic study that they can be recognized as being older than the meningeal lesions.

Subsequent studies attempted in a manner similar to that of Rich and McCordock (27, 28, and 29) have not always resulted in similar conclusions. Some investigators did not substantiate the high incidence of local extension to the meninges. Others (30, 31, 32) are in general agreement with Rich and McCordock.

The findings in the present investigation appear to support the views expressed by Rich and McCordock (22). Serial sectioning of the brain as performed by these investigators was not routinely carried out in the present series. Nevertheless, three-millimeter sections of the brain were made in 23 of the 108 cases of tuberculous meningitis in the group, of which 20 (86.9 per cent) showed the presence of tuberculous foci which could have been the source of the meningeal infection. Tuberculous foci were present in the brain parenchyma of the other 3 patients but they did not reach the meninges. Since careful sectioning of the brain was not carried out in the remaining cases of meningitis in the present series, sufficient material for a true evaluation was not obtained. Despite the fact that parenchymal foci were not searched for assiduously in all cases, 66 (61.1 per cent) of the 108 cases contained such foci. It was observed that 40 (37 per cent of the entire series) were contiguous with the meninges and may have caused the meningitis.

Rich and McCordock (22), in discussing the origin of the local caseous foci which give rise to the meningitis, believe that some are undoubtedly the result of the early dissemination of bacilli which occurs so commonly during the primary infection. Others develop from a dissemination as a result of reinfection. They believe that many of these foci become sterile. In others bacilli remain alive but, in the presence of acquired resistance, their ability to multiply is held in abeyance and the focus may remain quiescent for years to suffer a recrudescence of activity because of favorable local conditions.

It has been the experience of the present writer that, with rare exceptions, foci in the brain which become encapsulated are of no further danger in the development of meningitis. The foci which cause the meningitis progress toward the meninges from their onset. If the focus takes some time to reach the meninges, hematogenous foci of the same age within the other viscera may undergo anatomic healing. In those cases in which the foci develop from a primary complex which subsequently heals, the healing process may be so advanced that only occasional miliary foci remain as evidence of the original hematogenous seeding.

SUMMARY

Tuberculous meningitis was present in 42.2 per cent of a series of 97 fatal cases of tuberculosis in children and was usually related to a spread from a primary complex. In adults, tuberculous meningitis occurred in 2.9 per cent of

2,236 fatal cases of tuberculosis and was generally associated with an acute generalized miliary tuberculosis.

The difference between the pathogenesis of tuberculous meningitis may explain in part the better results obtained in children than in adults who have been treated with streptomycin. In children the source of dissemination is eliminated by the healing of the primary infection, whereas in adults the meningitis is generally secondary to an active extrapulmonary tuberculosis which gives rise to repeated hematogenous disseminations.

Sumario

Meningitis Tuberculosa: Correlación de los Resultados Terapéuticos con la Patogenia y las Alteraciones Anatomopatológicas: I. Consideraciones Generales y Patogenia

Hubo meningitis tuberculosa en 42.2 por ciento de una serie de 97 casos letales de tuberculosis en niños, estando por lo general enlazada con propagación de un complejo primario. En los adultos, hubo meningitis tuberculosa en 2.9 por ciento de 2,236 casos letales de tuberculosis, asociándose por lo general con granulia aguda.

Esa diferencia en la patogenia de la meningitis tuberculosa puede explicar en parte los mejores resultados obtenidos en los niños que en los adultos tratados con estreptomicina. En los primeros, el foco de difusión queda eliminado con la curación de la infección primaria, mientras que en los adultos la meningitis es generalmente secundaria a una tuberculosis extrapulmonar activa que produce repetidas diseminaciones hematógenas.

Resume

Méningite tuberculeuse: Corrélation des résultats thérapeutiques, de la pathogénèse et des modifications pathologiques: I. Considérations générales et pathogénèse

Dans la tuberculose de l'enfance, la méningite tuberculeuse a été observée dans 42,2% des cas dans une série de 97 cas mortels, celle-ci était habituellement consécutive à l'extension d'un complexe primaire. Chez l'adulte, la méningite tuberculeuse s'est manifestée dans 2,9% des cas dans une série de 2.236 cas mortels de la tuberculose; elle était habituellement associée à la tuberculose miliaire généralisée.

Cette différence dans la pathogénèse la méningite tuberculeuse peut expliquer en partie les meilleurs résultats obtenus chez les enfants que chez les adultes ayant été traités par la streptomycine. Chez l'enfant, la source de la dissémination est éliminée par la guérison de l'infection primaire, tandis que, chez l'adulte, la méningite est généralement secondaire à une tuberculose extra-pulmonaire qui donne lieu à une dissémination hématogène répétée.

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TUBERCULOUS MENINGITIS: CORRELATION OF THERAPEUTIC RESULTS WITH THE PATHOGENESIS AND PATHOLOGIC CHANGES[1, 2, 3, 4]

II. Pathologic Changes in Untreated and Treated Cases

OSCAR AUERBACH[5]

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Reports on the effect of streptomycin therapy on the pathologic changes of tuberculous meningitis have become quite numerous in the literature since the first reports of Baggenstoss, Feldman, and Hinshaw (1) and of Flory and his associates (2). There is general accord on the healing changes which occur after prolonged use of the drug. In a previous report by the present writer (3), observations concerning the pathogenesis of tuberculous meningitis were presented. In the present report an effort has been made to compare the gross and microscopic findings in treated and untreated cases of tuberculous meningitis in an attempt to correlate the evidences of therapeutic effect with the pathogenesis and pathologic changes of the disease.

Observations

Pathologic Anatomy in Untreated Cases of Tuberculous Meningitis

General Considerations

Gross appearance: In an untreated case of tuberculous meningitis, a gelatinous exudate lies in the subarachnoid space. Although the exudate is present from the pons to the optic nerves and to the lateral aspects of the brain, the greatest concentration is in the region of the optic chiasm. The exudate is present in the Sylvian fissures and in rare instances may extend to the convexities of the brain. In well-advanced cases the leptomeninges contain foci which measure up to 1 mm. in diameter. These follow the blood vessels and, although they are present over the lateral aspects of the cerebrum and cerebellum, they are most numerous and most often observed in the Sylvian fissures. Hektoen (4) stressed the general distribution of the miliary foci and the special predilection for the Sylvian arteries as characteristic of the blood-borne infection. These nodules are present in two locations: (*1*) on the inner surface of the arachnoid membrane and (*2*) as nodular swellings on the adventitial surface of the blood vessels.

[1] From the Laboratory Service, Halloran Veterans Administration Hospital, Staten Island 2, New York.

[2] Sponsored by Veterans Administration and published with approval of Chief Medical Director. The statements and conclusions published by the author are a result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[3] This study was made at Sea View Hospital while the author was pathologist at that institution.

[4] This paper was presented, in part, at the Tenth Streptomycin Conference of the Veterans Administration at Atlanta, Georgia, January 27, 1951.

[5] Home address: 121 Hamden Avenue, Staten Island 6, New York.

A similar gelatinous exudate containing round nodules often covers the choroid plexus within the lateral ventricles. The ependymal lining of the ventricle often has a granular surface if the meningitis has been present for some time.

The brain is generally heavier than normal in tuberculous meningitis and may increase its bulk by 25 per cent when the patients have had meningitis for two or three weeks. The fissure then tends to become obliterated and the convolutions are somewhat flattened. The lateral ventricles are slightly to moderately dilated. The dilatation generally increases with the duration of the disease.

Microscopic examination: The pathologic process in tuberculous meningitis is a fairly uniform one and varies only slightly from case to case. Lying between

FIG. 1. A liquefied caseous focus within the brain rupturing into an adjoining fissure. ×100.

the pial and arachnoid membrane is an extensive fibrin network, the meshes of which enclose a pink homogeneous fluid. This gives the exudate its gelatinous appearance when seen on gross inspection. A varying number of cells lie within this network; these are polymorphonuclear leukocytes, erythrocytes, and macrophages in the early stages. Lymphocytes become more numerous in the later phases of the disease. In focal areas, the exudate may have a caseous appearance. Nuclear fragments are found within these beginning caseous areas. Epithelioid cells and giant cells surround the older necrotic foci. The foci of caseation are more numerous toward the pial surface.

The changes noted in the blood vessels consist of cellular accumulations in the subendothelial region of the intima, nodular formation in the adventitia,

and fibrinoid changes of the media. The changes are not uniform from vessel to vessel. The process in the intima consists of endothelial cells intermingled with lymphocytes and occasional polymorphonuclear leukocytes. Generally the (infiltrating) cells are limited to the intima, but occasionally they may extend to the media. Within the adventitia are circumscribed tubercles composed of epithelioid cells and giant cells, often with a central zone of caseation. Occasionally these foci may extend into the media and they may sometimes reach the intima. These foci represent the small nodules seen along the course of the blood vessels in the base of the brain.

The fibrinoid transformation of the media is accompanied by a loss of nuclear structure. Less frequently this fibrinoid change is observed in the intima and occasionally also in the adventitia. It is best seen in smaller blood vessels. Hektoen (4) believed this to be a hyaline degeneration which occurs very rapidly under the influence of the acute inflammatory process.

Characteristic of untreated cases is the small amount of caseation and the slight tendency for the formation of granulation tissue.

PATHOLOGIC CHANGES IN CASES OF TUBERCULOUS MENINGITIS TREATED WITH STREPTOMYCIN

As a result of the effective use of chemotherapy, the pathologist has been introduced to a new phase of tuberculous meningitis.

MATERIAL

This portion of the study is based upon 4 cases of tuberculous meningitis at Halloran Veterans Administration Hospital, in which gross and microscopic studies were made by the writer, and 60 cases from the Armed Forces Institute of Pathology, in which the protocols and microscopic slides were studied.

Streptomycin therapy was administered by both intramuscular and intrathecal routes. The course of therapy varied from one day to twenty-five months. Unfortunately, the degree to which the infecting organisms were susceptible to streptomycin *in vitro* and the exact amount of streptomycin administered were not available. In most of the cases in which the drug was given over long periods of time (generally more than six months), there were intervals in the course of the disease in which no treatment was given.

Gross appearance: There were very characteristic changes which occurred in the meningitis under prolonged streptomycin therapy. The gelatinous character of the exudate had given way to a thickened dull gray mass which, because of the large amount of connective tissue within it, cut with increased resistance (figure 2). The subarachnoid space was obliterated by this dense mass of tissue. Enclosed within this gray layer, which in some instances reached a thickness of 1 to 2 cm., were yellow zones of caseation (figure 4). In some specimens this mass also covered the foramina of Luschka, obliterating the communication between the ventricles and the subarachnoid space (figure 3). On the convexities, especially over the sulci, the meninges were thickened by gray tissue. In some cases a gelatinous exudate was present in some sulci or surrounding the spinal cord. This was also noted in cases in which progression of the disease had occurred

after streptomycin treatment had been halted. The nodular thickenings along the course of the blood vessels, particularly in the Sylvian fissure, were generally absent, especially in those cases which had been under prolonged treatment. Slight gray thickenings of the adventitia were noted in some instances.

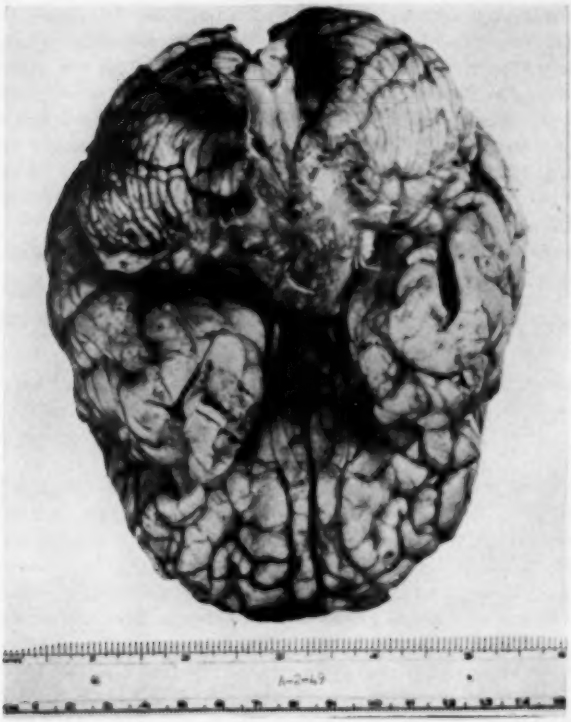

FIG. 2. Tuberculous meningitis in a patient who had received streptomycin therapy for seven months. The tuberculous process is concentrated over the brain stem and shows an abundance of connective tissue strands.

In general, cross-section of the brain showed a pronounced dilatation of the lateral ventricles, which was most advanced in those cases in which the foramina of Luschka were obliterated.

Microscopic appearance: The findings herein described are based on studies of autopsy material in which death was directly or indirectly due to the tuberculous meningitis.

Changes within the exudate: There were two characteristic changes which occurred within the fibrinocellular exudate, namely, a degree of caseation far in excess of that noted in untreated cases and marked proliferation of granulation tissue.

As the tuberculous process continued under streptomycin therapy, portions of the exudate apparently underwent caseation. These areas were of varying size and number and appeared to be related to the extent of the original exudate. Although the areas of caseation might have been noted in any part of the exudate, they were chiefly situated near the pia and in the region of the blood vessels

FIG. 3. (Top) Cross-section of the brain (figure 2) shows an obliteration of the foramina of Luschka by the organized tuberculous exudate at the base of the brain.

FIG. 4. (Bottom) Tuberculous meningitis after 25 months of streptomycin therapy. The medulla oblongata is surrounded by a very wide layer of hyalinized connective tissue containing areas of caseation.

(figure 5). In the latter areas the zones of caseation apparently represented a transformation of the exudate into a necrotic substance which might partially or completely surround the vessels. In many instances the adventitia in these regions was also involved by the caseous process (figure 6). These changes are different from those seen in untreated cases in that the adventitia was involved by the caseous process which commenced outside the vessel and extended toward the

lumen. The adventitial involvement in untreated cases is usually that of epithelioid-giant cell tubercles rather than broad zones of caseation. Less frequently the caseation extended into the media for varying depths. The intima was generally spared by the necrosis. Areas of caseation unrelated to the blood vessels were also present in the depth of the exudate.

Studies of cases in the earlier phases of treatment revealed that the granulation tissue apparently developed from the perivascular connective tissue, from the pia, and from the arachnoid and arachnoid trabeculae in the regions which had not undergone caseation. It contained typical epithelioid cells as well as fibroblasts. Langhans' giant cells, although present, were usually much less numerous than those customarily seen in tuberculous granulation tissue. The cellular elements formed wide collars of varied size around the blood vessels and differed from those seen in untreated cases in which foci are usually present in the adventitia in a single localized area (figure 7). In the treated cases, near the pia and the arachnoid, the epithelioid cells and fibroblasts extended along fibrin threads toward the depth of the exudate, and with this invasion there was a concomitant disappearance of the polymorphonuclear leukocytes. The granulation tissue apparently extended to surround and partially replace areas of caseation. Thus, the tuberculous meningitis in this stage of development showed areas of caseation intermingled with zones of granulation tissue as well as areas of the original fibrinocellular exudate.

As the process of healing continued, the collagen fibrils apparently increased until the granulation tissue was replaced by hyalinized connective tissue. Thus, in cases of tuberculous meningitis treated for more than one year, areas of caseation were surrounded by either granulation tissue containing abundant collagen fibrils or hyalinized connective tissue. When the process was of long standing (two years), most of the exudate was replaced by wide zones of hyalinized connective tissue.

Less advanced stages of healing were also present over the convexities in the cases in which there had been repeated exacerbations, and in these cases the granulation tissue was more cellular. A fibrinocellular exudate similar to that seen in untreated cases was also present in this region. It is possible that this lack of healing represented sites of reinfection. Since the bulk of the process was concentrated in the basal aspects, areas of more recent inflammation presumably would be more easily discerned in areas of minimal involvement.

Changes within the blood vessels: Concomitant with the transformation of the exudate around the blood vessels, there was a fibrous thickening of the intima of the enclosed structures with a corresponding decrease in the size of the lumen. In those regions in which the caseation only partially surrounded the vessel wall, intimal thickening occurred only on the side bordering the necrotic process. This obliterating endarteritis is a characteristic finding in blood vessels bordering zones of caseation (figure 6). For this reason it was seen with greater frequency in the lungs and was not so common a finding in the meninges until the advent of streptomycin.

The subendothelial cellular collections so frequently observed in the untreated cases were rarely seen in the cases which had been treated for a few months.

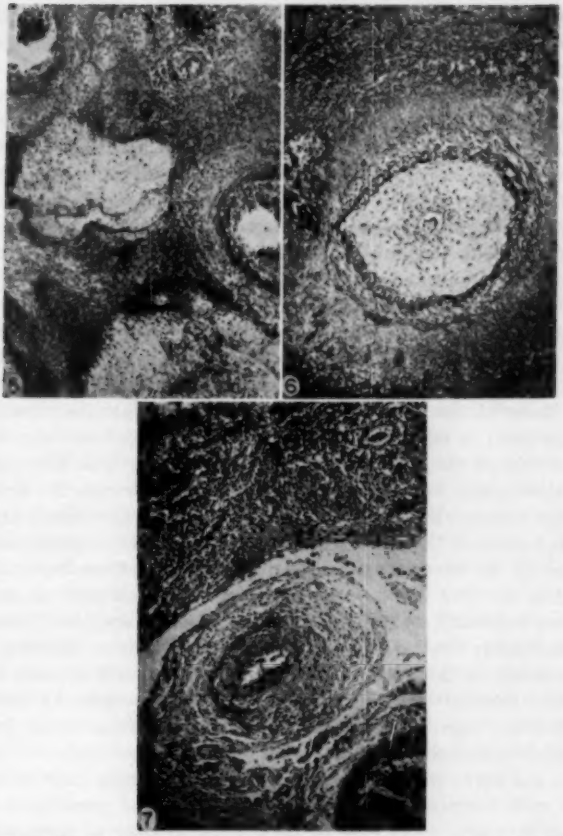

FIG. 5. A transformation of much of the exudate into caseous necrosis in a patient under streptomycin therapy for three months. ×48.

FIG. 6. Advanced intimal thickening of an artery in a patient under streptomycin therapy for six months. The caseous necrosis in the leptomeninges encroaches upon much of the vessel wall. ×48.

FIG. 7. A small blood vessel surrounded by a wide zone of granulation tissue in a case under streptomycin therapy for five months. The adjoining leptomeninges are occupied by granulation tissue containing an occasional Langhans' giant cell. ×48.

DISCUSSION

In the usual course of events, a tuberculous process within the serous cavities or the lungs is characterized by an exudate abundant in fibrin. The exudate differs from that caused by any pyogenic microorganism only in that tubercle bacilli are present. One of two possible things may occur to the exudate. It may undergo resolution and disappear or, more frequently, it may be transformed into a caseous mass.

In untreated tuberculous meningitis, the patient is usually dead of the disease within three weeks of its onset. In this stage of development the morphologic picture is that of a serofibrinous exudate with only small areas of caseation. The question now arises whether, at least in the cases of long duration, the exudate undergoes caseation and does not resolve during streptomycin therapy. This is what might be expected in untreated cases if the patient lived for a period beyond three weeks. It has been demonstrated that zones of frank caseation within the lungs, such as are found in the walls of cavities, are not affected by streptomycin therapy. There is little reason to expect that the tissue reaction in the meninges would differ greatly in this respect. The small central zones of caseation may furnish the invading microorganism with a safe harbor during therapy. When therapy is discontinued, such small nidi of infection would be freed of restraint and capable of further progression. The massive zones of caseation seen at the basal aspects of some of the brains could be accounted for on this basis. Strands of necrotic collagenous tissue which have the conformation of similar tissue at the periphery of partially healed foci are present in some of the larger areas of caseation. This suggests that they have been overrun by renewed activity on the part of the infection they contained. The question also arises whether resolution of the exudate can occur through the action of the streptomycin therapy. In the lungs, where an analogous process is present, it is assumed that much of the clearing of acute lesions under streptomycin therapy, as visualized in the roentgenograms, is due to the rapid resolution of the perifocal reaction (5). This is composed chiefly of serum, alveolar phagocytes, and erythrocytes, but no fibrin and polymorphonuclear leukocytes. It has been the writer's impression that the central pneumonic exudate, differing from the perifocal reaction in that it contains fibrin and polymorphonuclear leukocytes, was similarly capable of undergoing resolution. This may be an erroneous assumption if meningeal lesions undergo caseation while under therapy, as the large areas of caseation may indicate.

Levinson and his co-workers (6), in a study of 26 autopsy cases of tuberculous meningitis with streptomycin therapy, also noted the persistence of grossly caseous foci in nearly every case of their series in spite of adequate and prolonged streptomycin therapy. They also found that there is no evidence that the initial exudate is ever absorbed and that, if healing does occur, it does so by hyalinization or by fibrous walling-off of a still caseous zone.

In this respect, the findings of Cathie and McFarlane (7) are extremely interesting. These authors used streptokinase as an adjuvant to streptomycin in the treatment of tuberculous meningitis. This was given with the thought of promoting the lysis of the fibrinous exudate. They gave the streptokinase from the onset of treatment and found it of no value late in the disease. In a series of 20 patients (children) treated with streptomycin alone, 25 per cent survived, while in a series of 40 patients treated with streptomycin and streptokinase 58 per cent survived.

The presence of dense hyalinized connective tissue is due, in part, to the natural healing tendency of a tuberculous process of long duration but chiefly, perhaps, to the action of streptomycin.

The extensive fibroblastic proliferation within the intima of the vessels in the zone of caseation, also noted by other authors (8, 2, 9, 10, 11, 12, 13), is in sharp contrast to the untreated cases. The writer does not believe that these changes are necessarily related to the subendothelial cellular collections present in the untreated cases. Similar intimal thickening occurs in many vessels bordering a tissue which is undergoing necrosis.

Bunn (14) has pointed out a difference in the results in the treatment of meningitis in children and in adults. The figures for the mortality in the children are from 15 to 66 per cent, while in adults they vary from 50 to 90 per cent. He included only patients who were followed for at least five months after treatment was ended. This author gives us one of the possible explanations, that in children tuberculous meningitis in most instances is related to the onset of primary tuberculosis; while in adults meningitis usually develops in association with acute generalized hematogenous tuberculosis.

The present writer believes that the essential difference between the pathogenesis of tuberculous meningitis in children and in adults is that, in the former, meningeal infection usually results from the hematogenous dissemination from a primary complex in which the source of dissemination is eliminated by the healing of the primary infection. In adults, on the other hand, the meningitis is generally secondary to an active extrapulmonary tuberculosis which gives rise to repeated hematogenous dissemination.

Since repeated seedings to the meninges are not a factor in the course of tuberculous meningitis in children, the opportunity for successful results with streptomycin therapy would appear to be much greater than that in adults. That there are repeated infections of the meninges in the latter groups is suggested by the presence of all stages of development of the healing process and also by the presence of fresh exudate over the convexity of the brain and surrounding the spinal cord. Repeated relapses in the course of streptomycin therapy may be related to the appearance of fresh foci of inflammation in the subarachnoid space.

SUMMARY

A series of 64 cases of tuberculous meningitis treated with streptomycin therapy for variable periods of time has been studied. On the basis of these observations the following statements are permissible.

Streptomycin therapy has introduced a new phase of tuberculous meningitis. The drug prolongs the natural course of the disease and also results in extensive healing changes within the exudate.

The survival period of tuberculous meningitis in untreated cases is generally less than three weeks, too early for extensive caseation and granulation tissue to develop. Under streptomycin therapy the exudate within the subarachnoid space undergoes extensive necrosis which encircles and partially involves the vessel walls in a similar process. Concomitantly there is a fibrous thickening of the intima of the enclosed blood vessels. Tuberculous granulation tissue which develops from the pia arachnoid replaces the remaining fibrinocellular exudate in the subarachnoid space so that, in cases receiving prolonged therapy, the

base of the brain is covered by a dense layer of hyalinized connective tissue which encloses necrotic foci. The course of the tuberculous meningitis in cases which receive streptomycin therapy is similar to that in other serosal surfaces in which the patients have survived the disease for long periods of time.

Sumario

Meningitis Tuberculosa: Correlación do los Resultados Terapéuticos con las Alteraciones Patógenas y Anatomopatológicas: II. Alteraciones Anatomopatológicas en los Casos No Tratados y Tratados

Estudiada una serie de 64 casos de meningitis tuberculosa tratados con estreptomicina durante distintos períodos de tiempo, están justificadas las siguientes declaraciones a base de dichas observaciones.

La estreptomicinoterapia ha introducido una nueva fase en la meningitis tuberculosa. La droga prolonga la evolución natural de la dolencia, dando además por resultado extensas alteraciones curativas en el exudado.

En los casos no tratados, el período de sobrevivencia de la meningitis tuberculosa es en general de menos de tres semanas, o sea demasiado breve para la formación de extensa caseación y tejido de granulación. Con la estreptomicinoterapia el exudado en el espacio aracnoideo experimenta considerable esfacelamiento que circunda y envuelve parcialmente las paredes de los vasos en un proceso semejante. A la vez preséntase espesamiento fibroso de la íntima de los vasos sanguíneos afectados. El tejido tuberculoso de granulación derivado de la piamadre-aracnoides suplanta el restante exudado fibrinocelular del espacio subaracnoideo, de modo que, en los casos que reciben una terapéutica prolongada, la base del cerebro queda cubierta por una espesa capa de tejido conjuntivo hialinizado que encierra focos esfacelados. En los casos que reciben la estreptomicinoterapia, la evolución de la meningitis tuberculosa es semejante a la observada en otras superficies serosas cuando los enfermos han sobrevivido la enfermedad durante largos períodos de tiempo.

Resume

Méningite tuberculeuse: Corrélation des résultats thérapeutiques, de la pathogénèse et des modifications pathologiques: II. Manifestations pathologiques dans les cas traités et non traités

Etude d'une série de 64 cas de méningite tuberculeuse soumise à la streptomycinothérapie pendant une période variable. Sur la base de ces observations les déclarations suivantes sont permises:

La streptomycinothérapie a introduit en clinique une nouvelle phase de la méningite tuberculeuse. Cetté médication prolonge le cours naturel de la maladie et détermine d'apparition de signes de guérison dans l'exudat.

La période de survie des cas de méningite tuberculeuse, non traités, est en général inférieure à trois semaines, sa durée est trop courte pour permettre une caséification extensive ou la formation de tissu de granulation d'origine tuberculeuse Sous l'influence du traitement par la streptomycine, l'exudat de l'espace

sous-subarachnoidien est le siège d'un processus nécrotique qui s'étend a la périphérie des vaisseaux et envahit partiellement la paroi vasculaire. En même temps il se produit un épaississement fibreux au niveau de l'intima des vaisseaux intéressés. Le tissu de granulation qui se forme à partir de l'arachnoide remplace l'exudat fibrino-cellulaire de l'espace sous-arachnoidien de telle sorte que, dans les cas soumis à un traitement prolongé, la base du cerveau est recouverte d'une couche dense de tissu conjonctif hyalin qui enveloppe le foyer nécrosé. Le cours de la méningite tuberculeuse des cas traités par la streptomycine est le même que celui des autres surfaces séreuses où la période de survie a été prolongée.

Acknowledgment

The author wishes to express his thanks to the Armed Forces Institute of Pathology for the privilege of studying a portion of this material.

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CHEMOTHERAPY AS PROPHYLAXIS AGAINST SECONDARY INTESTINAL TUBERCULOSIS[1,2]

A Report of 150 Autopsies

IVAR KÄLLQVIST

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INTRODUCTION

In a previous report (1) an opinion was expressed that para-aminosalicylic acid (PAS) has such an excellent therapeutic effect on secondary intestinal tuberculosis that the prognosis *quoad vitam* is largely dependent on the outcome of the underlying pulmonary disease. Other writers (2–6) have reported similar favorable results with streptomycin and thiosemicarbazone. If this assumption is correct, the effect of such medication should be apparent in the post-mortem findings in the gastrointestinal tract of patients who, despite chemotherapy, died of pulmonary tuberculosis.

Before the advent of chemotherapy, tuberculous enterocolitis was a very common complication of pulmonary tuberculosis, and the literature contains many reports on its frequency at autopsy. Thus, Zahn (7) published a series of 1,875 post-mortem examinations of cases of pulmonary tuberculosis, covering the years 1876 to 1901. He found a total secondary enterocolitis incidence of 63.2 per cent, with 61.5 per cent in males and 66.6 per cent in females. Crawford and Sawyer (8) reported a series of 1,400 autopsies performed during the period 1921 to 1933. There were 950 cases of phthisis, 67.9 of which showed intestinal involvement; and 59.3 per cent, ulcerative lesions. Crawford and Sawyer also point out a racial factor in the frequency of this complication: ulcerative disease was found in 42.2 per cent of 462 white patients and in 87.5 per cent of 101 Negro patients.

Tisell (9) compiled a table of 14 statistical reports published prior to 1938 in different countries which showed an autopsy incidence of secondary intestinal tuberculosis varying from 40 to 90 per cent.

Medlar (10), describing 100 autopsies of fatal phthisis, reported 55 cases of enterocolitis, in 45 of which ulcerative lesions were found.

It is generally believed that conditions which lower bodily resistance predispose to tuberculosis. The effect of World War II in this respect is illustrated by several statistical surveys. Grundner-Culemann (11) noted a marked rise in mortality from tuberculosis in all age groups during the years 1940 to 1947 as compared with 1935 to 1938. Another German writer, Boehm (12), cites his own and other observations to show that the frequency of secondary intestinal tuberculosis rose sharply during the war and postwar years.

In a study from Norway recently published, Andersen (13) reports secondary enterocolitis in 52.9 per cent of 736 autopsies on 1,404 patients with fatal phthisis. Andersen's study is divided into three ten-year periods. In the first of these, 1918

[1] From Söderby Hospital, Uttran, Sweden.

[2] This investigation has been carried out with the help of a grant from the Swedish National Association against Tuberculosis.

to 1927, the incidence of intestinal involvement was 68.2 per cent of 201 autopsies; in the second, it was 61.1 per cent of 279; and in the third, 31.3 per cent of 256. The decrease is attributed partly to improvements in hygiene, nutrition, and therapy.

Of the writers who have reported good results with chemotherapy in secondary intestinal tuberculosis, a few have described a histologic picture of healing. A search of the literature, however, reveals no figures for the frequency of secondary intestinal tuberculosis since chemotherapeutics have come into widespread use. The writer has therefore considered it to be of interest to survey the findings in the routine autopsies carried out at a hospital for pulmonary tuberculosis over a period which may be said to mark the transition to chemotherapy. The results of this survey form the basis for the present report.

AUTOPSY MATERIAL

The material was furnished by the autopsies performed at Söderby Hospital (pulmonary tuberculosis hospital for the city of Stockholm) during the three years 1948 to 1950. Limitation of the investigation to this relatively short period may be considered to have ensured the greatest possible homogeneity in the material while permitting the inclusion of untreated control cases.

The total number of deaths during this period was 266, of which 234 were directly due to pulmonary tuberculosis. Eighty-four of these latter were not examined post mortem. The clinicians had considered that autopsy would not add anything of interest to the established diagnosis or the relatives had refused consent to the procedure.

The total number of autopsies was 177, but 27 of these have been excluded from the report because tuberculosis was not the primary cause of death. The study therefore comprises the post-mortem findings in 150 cases of fatal pulmonary tuberculosis, almost invariably cavitary in type.

The antimicrobial agents given were para-aminosalicylic acid,[1] streptomycin, and thiosemicarbazone. They were given separately or in combination, and certain minimal requirements were stipulated with regard to total dosage: for para-aminosalicylic acid, 30 days' therapy with 10 gm. daily; for streptomycin, 10 gm. with 1 gm. daily; and for thiosemicarbazone, 1.5 gm. in a daily dosage of 25 to 200 mg. The great majority of patients, however, had received quantities considerably in excess of these minimal requirements.

Of the 150 autopsy cases, 46 of the patients had at some time received chemotherapy. Twenty patients had been treated with PAS alone, 9 with streptomycin alone, and one with thiosemicarbazone alone. The remaining 16 patients had been given two or all of the preparations.

In table 1 are shown the number of admissions, the total deaths, and the number of deaths from tuberculosis and other causes during each of the three years. Of the 32 patients with a nontuberculous major pathology, 4 had no concurrent active tuberculosis. The primary causes of death in this group were as follows: cancer, 11 cases; surgical complications, 9; hepatic cirrhosis, one case; acute

[1] PAS, enteric-coated granules, made by Ferrosan Ltd., Sweden.

monocytic leukemia, one case; agranulocytosis, one; and nonspecific pneumonia, one case. Cardiovascular disease accounted for the 8 remaining deaths. Intestinal tuberculosis was found in only one of the 27 autopsies in this group—in the patient with acute monocytic leukemia. This case has previously been reported ((1), Case 22).

With the object of illustrating the selection of cases for autopsy, certain data from the total fatalities are presented in tables 2 and 3.

In table 2 may be seen the number of patients given chemotherapy in the autopsy cases and in those not examined post mortem.

TABLE 1

Admissions and Mortality Rate 1948–1950

	ADMISSIONS	TOTAL DEATHS	DEATHS FROM TUBERCULOSIS	DEATHS FROM OTHER CAUSES	
				Concurrent active pulmonary tuberculosis	No active tuberculosis
1948	982	135 (13.8%)	129 (13.1%)	5	1
1949	1,076	78 (7.2%)	61 (5.8%)	16	1
1950	1,105	53 (4.8%)	44 (4.0%)	7	2
Total.....	3,163	266 (8.4%)	234 (7.4%)	28	4

(%) = per cent of admissions.

TABLE 2

Deaths from Tuberculosis

	AUTOPSIES			NO AUTOPSY		
	Total	Chemotherapy	No chemotherapy	Total	Chemotherapy	No chemotherapy
1948	72	1	71	57	2	55
1949	51	24	27	10	3	7
1950	27	21	6	17	11	6
Total.....	150	46	104	84	16	68

In table 3 the distribution of both of the above groups according to age, sex, and treatment is presented. It is seen that the groups are subdivided into patients less than and more than fifty years of age, and that these subgroups are proportionately very similar in the cases with and without autopsy. There are, however, some discrepancies in the totals in the five-year divisions among the younger patients.

As the autopsies were almost all performed by the same pathologist, Dr. A. Bergstrand of the Karolinska Institute, Stockholm, the technique was uniform. Only macroscopic ulcerative lesions have been considered. Tuberculous lymph nodes, tuberculous peritonitis, or pre-ulcerative changes, e.g., reddened mucosa, have not been included in the analysis.

TABLE 3

Age Distribution

Patients examined post mortem

	AGE IN YEARS							TOTAL	AGE IN YEARS					TOTAL
	15–20	21–25	26–30	31–35	36–40	41–45	46–50		51–55	56–60	61–65	66–70	>70	
Men														
Treated	—	3	6	1	2	1	4	17	3	4	3	1	—	11
Untreated	—	6	5	10	6	7	7	41	7	9	7	7	1	31
Women														
Treated	—	3	4	2	2	1	3	15	1	1	—	1	—	3
Untreated	1	4	7	7	4	—	2	25	3	1	2	1	—	7
Total	1	16	22	20	14	9	16	*98*	14	15	12	10	1	*52*

Patients NOT examined post mortem

Men														
Treated	—	—	1	1	2	1	1	6	1	—	—	—	—	1
Untreated	3	4	3	2	7	8	1	28	6	5	7	3	1	22
Women														
Treated	—	1	—	4	3	1	—	9	—	—	—	—	—	—
Untreated	2	1	5	2	3	—	1	14	—	1	1	—	2	4
Total	5	6	9	9	15	10	3	*57*	7	6	8	3	3	*27*

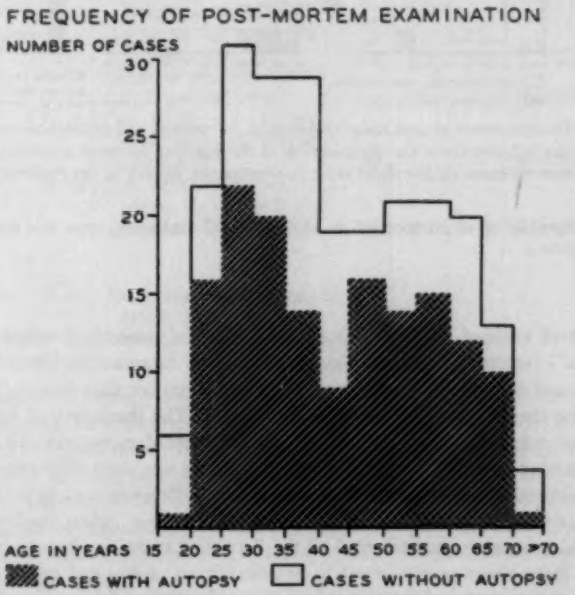

FIG. 1. The frequency of post-mortem examination in the five-year age groups of the total fatalities from pulmonary tuberculosis.

RESULTS

Untreated Cases

Secondary intestinal tuberculosis was found in 41 of the 104 untreated cases, i.e., in 39.4 per cent.

It is interesting to note that, despite the fact that the five-year groups in the male cases show no decline in mortality for patients more than fifty years of age, there was a striking reduction in the frequency of intestinal involvement in these older patients (figure 2). In 41 men aged twenty-one to fifty years, the frequency was 46.3 per cent as compared with 16 per cent in the 31 men over the age of fifty. The 25 women less than fifty years of age showed the high incidence of 60 per cent. The frequency figure for the women older than fifty was 28.6 per cent,

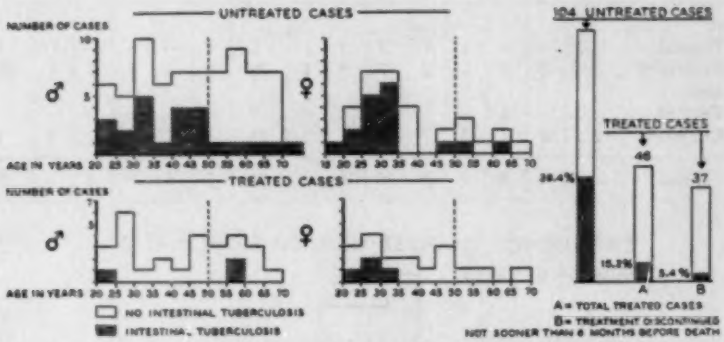

FIG. 2. The frequency of intestinal tuberculosis in treated and untreated cases. The four figures on the left illustrate the distribution of the autopsy material according to age and sex. The three columns on the right show the frequency figures in the respective groups.

but the number of older women in the study (7 patients) was too small for significance.

Treated Cases

Of the 46 treated cases examined post mortem, intestinal tuberculosis was present in 7 (figure 2). The complication had been diagnosed in life in 2 instances (Cases 2 and 3), and chemotherapy had been given for this reason. Fuller data concerning these 7 cases are supplied in table 4. The frequency of intestinal involvement was thus significantly lower in the treated cases, viz., 15.2 per cent. This reduction is statistically verified for patients less than fifty years of age.

Qualitative changes in intestinal lesions: The difference was most impressive, however, when the results were viewed *qualitatively*, i.e., when the lesions in the individual case were studied. In 6 of the 7 treated cases the lesions were totally different from those encountered in practically all of the untreated cases, both in regard to the extent of intestinal involvement and the dimensions of the ulcers.

Serpiginous ulcers, which were present in 63.3 per cent of the enterocolitis cases in the untreated group, were found in only one of the treated cases.

Interval between cessation of therapy and death: It is not to be expected that intestinal tuberculosis which has responded favorably to chemotherapy should thereafter remain unaffected by deterioration in the pulmonary condition, especially if chemotherapy is then withheld. Neither does chemotherapy given for pulmonary lesions confer immunity against intestinal involvement if medication is discontinued while the pulmonary process is still active. The interval between

TABLE 4

Chemotherapy Cases with Secondary Intestinal Tuberculosis

NUMBER	NAME	AGE AND SEX	INTESTINAL FINDINGS AT AUTOPSY	CHEMOTHERAPY (AMOUNT IN GRAMS)			PERIOD BETWEEN CESSATION OF DRUG AND DEATH
				PAS	Streptomycin	Thiosemicarbazone	
1	D. S.	F. 30	Small surface ulcers in terminal ileum and cecum	2,100	—	—	77 weeks
2	F. S.	M. 24	Healed and recent ulcers in cecum, ascending and transverse colon	410	—	—	66 weeks
3	M. E.	F. 34	Scar formation up to and including splenic flexure; recent scattered surface sores	1,190	—	—	53 weeks
4	I. O.	F. 22	Large, serpiginous ulcers in ileum and cecum	—	55	—	34 weeks
5	Y. L.	F. 27	One small, very recent ulcer in cecal mucous membrane	420	—	—	26 weeks
6	A. S.	M. 60	Two small, very recent ulcers in terminal ileum mucous membrane	1,170	46	—	17 weeks (PAS) 2 weeks (Streptomycin)
7	N. H.	M. 58	Numerous small, recent ulcers in terminal ileum	420	—	—	1½ weeks

the termination of therapy and death must therefore be taken into consideration when evaluating the post-mortem findings in the treated cases.

In table 4 it is shown that chemotherapy had been discontinued for more than one year prior to death in 3 cases and for more than six months in 2 cases.

Of the 37 cases in which not more than six months had elapsed between the discontinuation of chemotherapy and death, only 2 patients were found at autopsy to have intestinal tuberculosis. The frequency in these cases was therefore only 5.4 per cent (figure 2).

One case merits special description: A 26-year-old female with bilateral pul-

monary cavitation was treated with PAS for roentgenographically verified intestinal tuberculosis approximately eighteen months before her death ((1), Case 9). She later received all three chemotherapeutics in an endeavor to control her pulmonary disease, and medication was discontinued only four weeks before she died. Autopsy showed no macroscopic or microscopic signs of active intestinal tuberculosis although in places there were only insignificant remnants of gland ducts covered with a granulation tissue containing few connective cells.

TABLE 5

Statistical Analysis with Fisher's Exact Method

Males up to 50 years of age

	Final phase of activity					
	Less than 1 year			More than 1 year		
	NO intestinal tuberculosis	Intestinal tuberculosis	Total	*NO* intestinal tuberculosis	Intestinal tuberculosis	Total
Untreated cases*	10	11	21	10	8	18
Treated cases	6	—	6	10	1	11
Total	16	11	27	20	9	29
	$P = 0.027$			$P = 0.048$		

Females up to 50 years of age

Untreated cases	4	4	8	6	11	17
Treated cases	5	—	5	6	4	10
Total	9	4	13	12	15	27
	$P = 0.098$			$P = 0.150$		

* No history available in 2 cases.

Statistical Analysis

The frequency of intestinal tuberculosis in the treated and untreated cases was tested with the chi-square test. For *men aged twenty-one to fifty years*, the chi-square value was 7.008 $P < 0.01$. This implies that the complication was significantly less frequent in the treated cases. The test failed to show significance for men more than fifty years of age or for women.

On the advice of the statisticians, the material was subjected to further division. It was decided to divide the cases according to the duration of the final active phase of the pulmonary disease. This phase was estimated to begin with roentgenographic evidence of fatal progression or, where roentgenograms were not available, with hemoptysis or other symptomatic manifestations. It was considered possible that cases with a long final phase of activity might show a higher frequency of intestinal tuberculosis than those in which the duration of activity was shorter. Final activity was calculated in months, and the statisticians found a suitable dividing line at one year. The statistical analysis is presented in table 5.

The P-values for *men aged twenty-one to fifty years* show that the incidence of secondary intestinal tuberculosis is significantly lower in the chemotherapy group.

For *women less than fifty years of age*, the probability figures show that the difference is not fully verified if a significance level of 5 per cent is used. But as the variations point in the same direction, the P-values for short and long activity can be combined into a joint test by making the transformation $T = 2 \cdot \log \frac{1}{P}$. This gives a total value (T) corresponding to a probability of approximately 0.08. As the figures for such a combined value are always somewhat too high, it is possible that they, after correction, give the above-mentioned significance level of 5 per cent.

The statistical analysis shows, therefore, that chemotherapy has a *prophylactic* as well as a therapeutic action against secondary intestinal tuberculosis in males less than fifty years of age. Although the figures are not fully statistically verified for women in this age group, there can be very little doubt that here also chemotherapy played a prophylactic role.

In persons more than fifty years of age, the effect of chemotherapy on the frequency of secondary intestinal tuberculosis could not be demonstrated statistically.

DISCUSSION

As many writers have emphasized, clinical observations form an unreliable basis for the diagnosis of secondary intestinal tuberculosis. Gastrointestinal symptoms are frequently present in pulmonary tuberculosis and do not necessarily indicate intestinal involvement. On the other hand, there may be extensive intestinal disease with few or no symptoms. Even with the aid of contrast roentgenograms, it may be difficult to establish a diagnosis, especially if the lesions are confined to the small intestine. Post-mortem findings undoubtedly offer the greatest accuracy in the estimation of the frequency of this complication in fatal phthisis.

Viewed from a statistical angle, the autopsy material here presented invites the following comment: Analysis of the case sampling shows a random selection from the total phthisis fatalities but with the treated cases significantly more numerous in the autopsy material.

It is not surprising, however, that the post-mortem findings were considered of greater interest in the treated cases. In chemotherapy a factor was introduced which often has an amazing influence on the process of healing.

Of the 62 treated cases, 46 (74.2 per cent) were examined post mortem, as against 104 (60.5 per cent) of the 172 untreated cases. Since the chances of revealing intestinal disease were correspondingly greater in the treated cases, this cannot be regarded as prejudicial to the impartiality of the investigation; nor is there any reason to believe that the autopsies in the untreated cases were mainly carried out on subjects suspected of having intestinal tuberculosis. Moreover, the frequency in the untreated cases, 39.4 per cent, is not high in comparison with the figures reported in the literature. It is sometimes erroneously stated in the literature that the frequency of intestinal tuberculosis at this hospital for the years 1936 and 1937 was 88.5 per cent. This was the figure reported by Tisell (9), but his material was selected and was not claimed to be representative.

To obtain accuracy in a comparison such as that made in the present study,

it is obviously necessary to exclude any tendency towards a general decline in the frequency of secondary intestinal tuberculosis. Andersen (13) showed a fall in the incidence of this complication from 68.2 per cent in the decade 1918 to 1927 to 31.3 per cent in the decade 1938 to 1947. Andersen's material consisted of 736 autopsies on 1,404 cases of fatal phthisis. He has not accounted for the method of selection of cases for autopsy, and the frequency figures are therefore perhaps open to doubt. The downward trend, however, probably does exist. But even granted this decrease over thirty years, it cannot have been so rapid in the three years covered by this study as to be alone responsible for the fall from *39.4 per cent* in the untreated cases to *15.2 per cent* and *5.4 per cent* in the cases given chemotherapy.

With the object of investigating a possible downward trend among the untreated cases in the present study, the frequency figures for intestinal tuberculosis have been estimated for each of the three years (table 6). No decrease was apparent in 1949 and the figures for 1950 were too small for significance.

In addition to accomplishing the primary aim of determining the prophylactic value of chemotherapy against secondary intestinal tuberculosis, this investigation brought to light another interesting fact. As seen in the report on the untreated cases, the enterocolitis frequency in males more than fifty years of age was very low as compared with that in younger males. The number of women older than fifty was too small to permit such a comparison. This scarcity of older women is in conformity with the known fact that tuberculosis is relatively rare in such subjects. Rubin (14) has reported a series, unspecified as to sex, in which 324 patients aged twenty to fifty years showed a post-mortem enterocolitis frequency of 70.3 per cent; 95 patients aged fifty-one to eighty years had an incidence of 39 per cent. Information in the literature is scanty with reference to this age distribution of secondary intestinal tuberculosis.

TABLE 6

Post-Mortem Frequency of Intestinal Tuberculosis in Untreated Patients

YEAR	NUMBER OF AUTOPSIES	INTESTINAL TUBERCULOSIS
1948	71	28 = 39.4%
1949	27	11 = 40.7%
1950	6	2 = 33.3%

SUMMARY

A study of 150 autopsies on 234 cases of fatal phthisis at Söderby Hospital during the three-year period 1948 to 1950 is reported. The primary aim of the study was to determine whether or not chemotherapeutics specific for *M. tuberculosis* (para-aminosalicylic acid, streptomycin, and thiosemicarbazone) have a *prophylactic* action against secondary intestinal tuberculosis.

Of 104 cases *without chemotherapy*, 41, or *39.4 per cent*, were found to have ulcerative intestinal tuberculosis. Male patients less than fifty years of age had

an enterocolitis frequency of *46.3 per cent.* Men older than fifty showed a frequency of only *16 per cent.* In women less than fifty years of age, the incidence of intestinal involvement was *60 per cent.* The number of women older than fifty was too small to permit reliable frequency figures.

Of 46 cases *with chemotherapy*, ulcerative intestinal tuberculosis was found in 7, or *15.2 per cent.* In 37 of these patients, chemotherapy had been discontinued less than six months prior to death. In this group only 2, or *5.4 per cent*, had intestinal involvement. The lesions in the untreated cases were much more extensive than those in the treated cases. Serpiginous ulcers were found in *63.3 per cent* of the enterocolitis cases in the former group and in only *14.3 per cent* of the latter.

The frequency figures are statistically significant for men less than fifty years of age; the figures for women less than fifty lie well within the bounds of statistical probability.

The study shows, therefore, that antimicrobial therapy has effected a considerable decline in the frequency of ulcerative enterocolitis in fatal phthisis. Antimicrobial agents specific for *M. tuberculosis* have thus a *prophylactic* action against secondary intestinal tuberculosis.

SUMARIO

La Quimioterapia como Profilaxis Contra la Tuberculosis Intestinal Secundaria. Presentación de 150 Autopsias

Este estudio abarca 150 autopsias realizadas en 234 casos de tisis letal tratados en el Hospital Söderby durante el trienio 1948–1950. Fué fin primordial del estudio determinar si las quimioterapias específicas para el *M. tuberculosis* (ácido para-aminosalicílico, estreptomicina y tiosemicarbazona) ejercen o no efecto profiláctico contra la tuberculosis intestinal secundaria.

De 104 casos atendidos sin quimioterapia, 45, o sea 39.4 por ciento, mostraron tuberculosis intestinal ulcerada. En los varones de menos de 50 años, la enterocolitis alcanzó una frecuencia de 46.3 por ciento. En los de más de 50 años, la frecuencia no pasó de 16 por ciento. En las mujeres de menos de 50 años, la incidencia de compromiso intestinal llegó a 60 por ciento. El número de las de menos de 50 años fué insuficiente para poder sacar cifras aceptables en cuanto a frecuencia.

De 46 casos atendidos con la quimioterapia, 7, o sea 15.2 por ciento, tenían tuberculosis intestinal ulcerada. En 37 de estos enfermos, la quimioterapia había sido discontinuada menos de seis meses antes del fallecimiento. En este grupo, sólo 2, o sea 5.4 por ciento, mostraban afección intestinal. En los casos no tratados, las lesiones eran mucho más extensas que en los tratados, encontrándose úlceras serpiginosas en 63.3 por ciento de los casos de enterocolitis del primer grupo y sólo en 14.3 por ciento de los del segundo grupo.

Las cifras de la frecuencia son estadísticamente significativas para los hombres de menos de 50 años, y para las mujeres de dicha edad quedan bien dentro de los límites de las probabilidades estadísticas.

Este estudio demuestra, por consiguiente, que la quimioterapia ha logrado una baja considerable en la frecuencia de la enterocolitis ulcerada en la tisis letal. Los agentes quimioterapéuticos específicos para el *M. tuberculosis* ejercen, pues, efecto profiláctico contra la tuberculosis intestinal secundaria.

RESUME

La chimiothérapie comme mesure de prophylaxie contre la tuberculose intestinale secondaire. Un rapport d'après 150 autopsies

Ce rapport concerne 150 autopsies effectuées à la suite de 234 décès par tuberculose pulmonaire au "Söderby Hospital" pendant une période de trois années (1948–1950). Le but principal de cette étude était de déterminer si la chimiothérapie spécifique contre le bacille tuberculeux (acide para-aminosalicylique, streptomycine, et thiosemicarbazone) exerçait une action *prophylactique* contre le tuberculose intestinale secondaire.

Dans 104 cas non soumis à la chimiothérapie, la tuberculose intestinale ulcéreuse a été observée 41 fois, soit dans 39,4% des cas. L'incidence de l'entérocolite chez les hommes âgés de moins de 50 ans était de 46,3%. Au-dessus de 50 ans elle n'était que de 16%. Chez les femmes âgées de moins de 50 ans, l'incidence de lésions intestinales secondaires était de 60%. Le nombre de femmes plus âgées était trop réduit pour établir une statistique valable.

Parmi 46 malades soumis à la chimiothérapie, 7, soit 15,2%, présentaient des lésions de tuberculose intestinale ulcéreuse. Chez 37 d'entre eux, la chimiothérapie avait été discontinuée moins de six mois avant le décès. Dans ce groupe l'intestin n'était lésé que dans 2 cas, soit 5,4%. Les lésions étaient plus étendues dans les cas non traités que dans les cas traités. Des ulcères serpigineux ont été constatés dans 63,3% des cas d'entéro-colite du premier groupe et dans seulement 14,3% des cas dans le second groupe.

En ce qui concerne les hommes âgés de moins de 50 ans, l'incidence observée a une valeur statistique; les chiffres relatifs à l'incidence chez les femmes de moins de 50 ans paraissent dans les limites des probabilités statistiques.

En conséquence, cette étude démontre que la chimiothérapie a considérablement réduit l'incidence de la tuberculose intestinale ulcéreuse observée dans les cas mortels de tuberculose pulmonaire. Les agents chimiothérapeutiques exerçant une action spécifique contre le bacille tuberculeux ont ainsi une action prophylactique contre la tuberculose intestinale secondaire.

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ATTEMPTS TO HEIGHTEN THE EFFECT OF BCG VACCINATION WITH HYALURONIDASE[1, 2, 3]

SVEN BERGQVIST

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INTRODUCTION

In connection with investigations into the significance of multiple infection in tuberculosis which are in progress at St. Göran's Hospital, interest was aroused in the effect produced by hyaluronidase, particularly in association with *Staphylococci* (1–5).

In a previously reported investigation into the effect of a complicating infection on the course of experimental tuberculosis in animals, it was demonstrated that guinea pigs infected simultaneously with tubercle bacilli and *Staphylococci* lost weight more rapidly, had a shorter survival time (5), and reacted sooner and more strongly to tuberculin (6) if the *Staphylococci* were active hyaluronidase producers than if they produced little or no enzyme. This result suggested the possibility of attempting to improve the effect of BCG vaccination by adding hyaluronidase to the vaccine. Tests on guinea pigs carried out along these lines, and already described in an earlier publication (7), were successful in producing a more rapid and stronger tuberculin reaction after the addition of hyaluronidase to the vaccine. As was to be expected, a larger papule developed after the vaccination but, on the other hand, ulceration was not any more frequent than it was in animals inoculated with vaccine alone. The experiments were carried out in two series, 1 mg. of BCG bacilli being used in one case, and 5 mg. in the other, with uniform results in both groups. As a result of these observations in guinea pigs, it was decided to investigate them in man.

MATERIALS AND METHODS

In October 1950, a series of schoolchildren in Stockholm[4] were vaccinated. BCG vaccine which contained hyaluronidase was used in one group of children, and BCG vaccine alone in another group. Boys and girls in the second class at elementary schools in the Kungsholm district were subjected to a tuberculin test, first with ointment (Moro) and then, if the result was negative, a Mantoux test with 1 mg. of Old Tuberculin. Among 211 tuberculin-negative children, the parents' permission to carry out BCG vaccination in 178 was obtained; and of these, 100 (45 boys and 55 girls) were vaccinated with BCG vaccine containing hyaluronidase. The remaining 78 children (38 boys and 40 girls) were vaccinated

[1] From the Medical Tuberculosis Department, St. Göran's Hospital, Stockholm, Sweden.

[2] Aided by grants from the National Antituberculosis Association of Sweden.

[3] Presented before a meeting of the Swedish Tuberculosis Physician Association, March 31, 1951.

[4] This was made possible through the courtesy of Dr. Urban Hjärne, chief medical inspector of schools.

in the usual manner, without hyaluronidase. Of the first group, 7 had been vaccinated previously as had 2 in the control group.

Preparation and administration of vaccine: Five milliliters of BCG[4] vaccine (containing 0.5 mg. of bacilli per ml.) were transferred to an ampoule with 25 viscosity-reducing units (V.R.U.) of hyaluronidase (Hyalas "Leo"). Hyalas[5], which is a purified and vacuum-dried enzyme from bull's testes, forms a thin, hardly noticeable film on the bottom of the ampoule and therefore has no measurable effect on the volume of the vaccine administered. The vaccination was done intracutaneously on the upper part of the arm in the boys and on the thigh in the girls. The amount of vaccine injected was 0.1 ml. (0.05 mg.) and this contained, as may be seen from the above, 0.5 V.R.U. of hyaluronidase.

RESULTS

The first tuberculin test was carried out in the form of a Mantoux test, 1 mg., twenty-six days after vaccination, and was interpreted three days later.

Of the children who were vaccinated with an addition of hyaluronidase, 57 were positive to tuberculin. Owing to illness in 6 instances, either the tuberculin test could not be carried out or it was not possible to study the reaction. Of the 78 children comprising the control group (BCG vaccination without hyaluronidase), 71 presented themselves for a check-up of their tuberculin reaction. Among these, 25 reacted positively to tuberculin. Thus, of 94 children observed in the hyaluronidase group, 57 were positive twenty-nine days after vaccination, or 61 ± 5 per cent. In the control group, on the other hand, only 25 of 71 children were positive, or 35 ± 5.7 per cent. The difference between these two percentages is 26, with a standard error of ± 7.6 per cent; in other words, a statistically significant difference.

The tuberculin tests were continued in those children who had been negative at the above-mentioned examination, and forty-seven days after vaccination a new tuberculin test was done. On this occasion, a cutaneous test, with ointment, was done first; and, if this also was negative, the 1 mg. Mantoux test was applied. In the hyaluronidase group, 24 of the 33 percutaneous tests were interpreted and 14 of the 15 Mantoux tests were positive. In the control group, 34 of the 47 Moro tests observed were interpreted as positive reactions to tuberculin, and of 12 Mantoux tests one-half were similarly positive.

Surveying the results (table 1), it is seen that 96 of 100 children who were vaccinated with BCG supplemented with hyaluronidase were tested with tuberculin fifty days after vaccination. All except one of these reacted positively at this examination (99 per cent). In the control group, which consisted of 78 vaccinated children, 71 were tested with tuberculin at the same time as the other group and 65 were found to be positive reactors (91 per cent). It may be added here that tests with tuberculin carried out in January 1951 on children who had previously showed a negative reaction, or who were not previously tested, all yielded positive reactions.

Local reaction to BCG: The local reaction to BCG vaccination was studied

[4] The BCG vaccine used is prepared by the Bacteriological Laboratory, the Sahlgren Hospital, Gothenburg, Sweden.

[5] Proprietary name of a preparation made by The Leo Co., Hälsingborg, Sweden.

for the first time twenty-nine days after inoculation. It was observed that it was slightly larger in the group of children who had received vaccine plus hyaluronidase. In 9 instances, a small ulceration was observed among these children, and in the control series 12 cases of ulceration were encountered. After the lapse of a little more than three months, the local reactions were examined again; information is furnished in table 2 regarding the size of the reactions and ulcerations. It will be seen from the table that the infiltrates were on an average larger in the

TABLE 1

Positive Reaction to Tuberculin in Per Cent of Total Subjects Studied Twenty-Nine and Fifty Days After BCG Vaccination With and Without Hyaluronidase

	MANTOUX 1 MG. OLD TUBERCULIN	MORO	MANTOUX 1 MG. OLD TUBERCULIN
Days after vaccination	29	50	82
BCG	35	83	91
BCG with hyaluronidase	61	87	99

TABLE 2

Size of Local Reaction Among Boys and Girls Vaccinated With BCG Vaccine With and Without Hyaluronidase

	LOCAL REACTION, DIAMETER IN MM.									
	BCG with hyaluronidase					BCG				
	≤5	10	15	>15 mm.	Total	≤5	10	15	>15 mm.	Total
Boys										
Infiltrates	6	29	5	2	42	22	11	—	—	33
Ulcerations	5	4	—	—	9	1	1	—	—	2
Girls										
Infiltrates	5	25	10	13	53	9	24	6	1	40
Ulcerations	4	11	3	1	19	3	4	—	—	7
Total										
Infiltrates	11	54	15	15	95	31	35	6	1	73
	12 per cent	57 per cent	31 per cent			42 per cent	48 per cent	10 per cent		
Ulcerations	9	15	3	1	28	4	5			9

hyaluronidase group[7] than in the control group. The percentage of ulcerations was two and one-half times as high in the hyaluronidase group as in the control series; in the latter group, also, the ulcerations were only small. In 4 girls in the hyaluronidase group, necrosis larger than 10 mm. in diameter appeared, the largest of these areas measuring 20 by 13 mm. A secondary infection, which soon healed after the application of ointment containing a quaternary ammonium com-

[7] Large infiltrates of 15 mm. or more in diameter were three times as frequent in this group.

pound, occurred in 2 of the cases. As in the case of other investigations into the occurrence of vaccination-necrosis (8, 9), the girls in this group were also more affected in this respect; this seems to have some connection with the fact that, in girls, the vaccination is done on the leg where the clothes rub and press on the sore and where there is a greater risk of secondary infection.

DISCUSSION

Judging by the results already gained in this investigation, a positive reaction to tuberculin occurred more rapidly when hyaluronidase (spreading factor) had been added to BCG vaccine than when vaccination was carried out in the customary manner (the difference was statistically significant). It is of great value at each vaccination to obtain a change to a tuberculin-positive reaction as soon as possible after inoculation. In certain cases, also, a person who has been vaccinated against tuberculosis needs to be declared fit as soon as possible to enter a tuberculous environment, and in such cases the method with the addition of hyaluronidase seems to be especially suitable. While a direct connection between immunity and tuberculin sensitivity by no means exists in general, the tuberculin-positive reaction is, in practice, regarded as being the sign that the protection provided by BCG bacilli has come into force. It still remains to be established by further study what degree of immunity was present a month after vaccination in the group of children, nearly two-thirds of the total, who already had a change in the tuberculin reaction at that time.

The observation time in this study is too short to allow any conclusions to be drawn concerning whether the new vaccination method is capable of causing a more permanent state of tuberculin hypersensitivity and a higher degree of immunity. However, Törnell (10) and, later, Hertzberg (11) have shown that, in general, a positive response to tuberculin lasts longer when the local reaction has been large and that the frequency and degree of tuberculous manifestations are, furthermore, lower in these cases. Since a larger local reaction is usually obtained when hyaluronidase is added, it is possible that the new method of vaccination might bring about an improvement in regard to both degree and duration.

It is not yet possible to decide whether the incidence of necrosis will continue to be higher in the hyaluronidase group in connection with later vaccinations. The difference between the number of cases of necrosis in both groups is, in fact, not statistically significant. In comparable statistical studies (9, 12), fairly large areas of necrosis are reported in 2 to 4 per cent of persons vaccinated with the ordinary technique, and the incidence of necrosis observed in the hyaluronidase group (4 per cent) therefore can hardly be said to be remarkably high. It should be mentioned, also, that hyaluronidase as such has no side-effects whatsoever, even if it is given in doses a thousand times greater than the one used in the present investigation.

SUMMARY

Ninety-five schoolchildren approximately eight years old were vaccinated with BCG vaccine to which hyaluronidase had been added, and a control series of 71 children were vaccinated in the usual manner. A tuberculin test (Mantoux, 1 mg.

of Old Tuberculin) made twenty-nine days later revealed that 61 per cent of those children who had received vaccine together with hyaluronidase were positive reactors, compared with 35 per cent of the control series. The difference, 26 per cent, is statistically significant.

Fifty days after vaccination, 99 per cent were tuberculin positive in the hyaluronidase group compared to 91 per cent in the control group.

The reaction to the vaccination was, in general, stronger among the children who had received hyaluronidase. An ulceration larger than 10 mm. in diameter developed in 4 of these children. No ulcerations of this size arose in the control group. However, the question of whether the uneven ulceration distribution in the two groups is definitely related to the hyaluronidase administration or perhaps results from some other unknown factor requires further elucidation.

The chances of being able to heighten the effect of BCG vaccination, with respect to both degree and duration, by adding hyaluronidase to the vaccine are discussed.

SUMARIO

Esfuerzos Encaminados a Realzar el Efecto de la Vacunación BCG con Hialuronidasa

Noventa y cinco escolares de unos ocho años de edad fueron vacunados con vacuna BCG a la cual se había agregado hialuronidasa, vacunándose en la forma habitual como testigos a otros 71 niños. Una prueba con tuberculina (Mantoux 1 mg.) hecha a los veintinueve días reveló que 61 por ciento de los niños que habían recibido vacuna con hialuronidasa eran positivos, comparado con 35 por ciento en la serie testigo. La diferencia, 26 por ciento, es estadísticamente significativa.

A los cincuenta días de la vacunación, 99 por ciento eran tuberculino-positivos en el grupo de la hialuronidasa, comparado con 91 por ciento en el grupo testigo.

La reacción a la vacunación fué, en general, más poderosa entre los niños que recibieron hialuronidasa. En 4 de esos niños se presentó una úlcera de más de 10 mm. de diámetro. En el grupo testigo no hubo úlceras de dicho tamaño. No obstante, necesita más dilucidación el punto de si la desigual distribución de las úlceras en los dos grupos está netamente enlazada con la administración de hialuronidasa o quizás proceda de algún otro factor desconocido.

Discútense las posibilidades de realzar el efecto de la vacunación BCG, en cuanto tanto a intensidad como duración, agregando hialuronidasa a la vacuna.

RESUME

Essais tendant à augmenter l'effet de la vaccination par le BCG par l'adjonction d'hyaluronidase

Quatre-vingt quinze écoliers âgés en moyenne de huit ans ont été vaccinés avec du vaccin BCG auquel de l'hyaluronidase avait été ajouté. Un groupe témoin composé de 76 enfants a été vacciné de la manière habituelle. Le tuber-

culin-test (Mantoux 1 mg.) pratiqué vingt-neuf jours plus tard a donné une réaction positive chez 61% des enfants ayant été vaccinés avec le vaccin contenant l'hyaluronidase, par opposition aux 35% du groupe témoin. La différence, soit 26%, représente une valeur statistique.

Cinquante jours après la vaccination, 99% du groupe ayant reçu l'hyaluronidase étaient positifs par rapport aux 91% de la série témoin.

La réaction vaccinale était en général plus forte chez les enfants ayant reçu l'hyaluronidase. Une ulcération de plus de 10 mm. de diamètre s'est produite chez 4 de ces enfants. Aucune ulcération de cette importance ne s'est manifestée dans le groupe témoin. Toutefois, la question de savoir si la distribution inégale qui s'est produite dans les deux groupes est absolument dépendante de l'administration de l'hyaluronidase, ou si elle est due à un facteur inconnu, demande à être élucidée.

La possibilité d'accroître l'effet de la vaccination par le BCG du point de vue de son intensité et de sa durée par l'adjonction d'hyaluronidase est étudiée.

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PLASMA CONCENTRATIONS OF PARA-AMINOSALICYLIC ACID (PAS) AFTER ORAL AND RECTAL ADMINISTRATION AS INFLUENCED BY *p*-(Di-*n*-Propylsulfamyl)-BENZOIC ACID (BENEMID)[1]

CHARLES W. RIEBER, MYRON SALINE, AND MAX M. FRIEDMAN

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INTRODUCTION

Para-aminosalicylic acid (PAS) is now accepted as a useful chemotherapeutic agent in the management of tuberculosis. The usual dosage by mouth is 8 to 12 gm. per day. The difficulty encountered in giving more than 8 gm. per day, because of annoying gastrointestinal symptoms, is well known. The sodium salt, although better tolerated, is more rapidly excreted and even in this form causes nausea, gastric distress, loss of appetite, and occasionally diarrhea in a significant number of patients. In a previous study (1), the absorption of sodium para-aminosalicylate from the rectum was investigated with the possibility that this might provide an alternative route for those patients who were unable to take the drug by mouth. The results of this study indicated that large doses, up to 12 gm. at a time twice a day, could be given by rectum without causing any symptoms. However, in comparing plasma concentrations after administration of sodium PAS by mouth with that by rectum, it was found that the concentrations obtained by the rectal route were one-half to one-third of those obtained by the oral method. This was particularly evident at two and four hours after administration of the drug. The plasma concentrations after 12 gm. by rectum were similar to those after 6 gm. by mouth. At the time that this work was being completed the studies by Boger and associates (2, 3, 4, 5) on the influence of *p*-(di-*n*-propylsulfamyl)-benzoic acid (Benemid)[2] on the plasma concentrations of PAS began to appear in the literature. These authors indicated that significant increases in PAS plasma concentrations could be obtained by the addition of Benemid without risk of toxicity from that drug. Accordingly, a study has been conducted in which a comparison was made of the plasma concentrations of PAS attained when the drug was administered by the oral or the rectal route, with and without Benemid.

METHOD

Six subjects were selected from the patients on the female tuberculosis service. All other medications were discontinued, including regular PAS therapy. In these patients, previous routine laboratory examinations had shown no evidence of impairment of kidney function. As in the previous investigation, a twenty per

[1] From the Medical Service of the Triboro Hospital and the Department of Laboratories of the Queens General Hospital, Jamaica, New York.

[2] Benemid is the proprietary name for *p*-(di-*n*-propylsulfamyl)-benzoic acid manufactured by Sharp & Dohme. The authors are indebted to Sharp & Dohme, Inc. for supplying the drug for this study.

cent solution of sodium para-aminosalicylate ('Parasal' Sodium)[2] was used. Benemid was given as a 0.5 gm. tablet by mouth every six hours for six doses beginning twenty-four hours prior to sodium PAS administration.

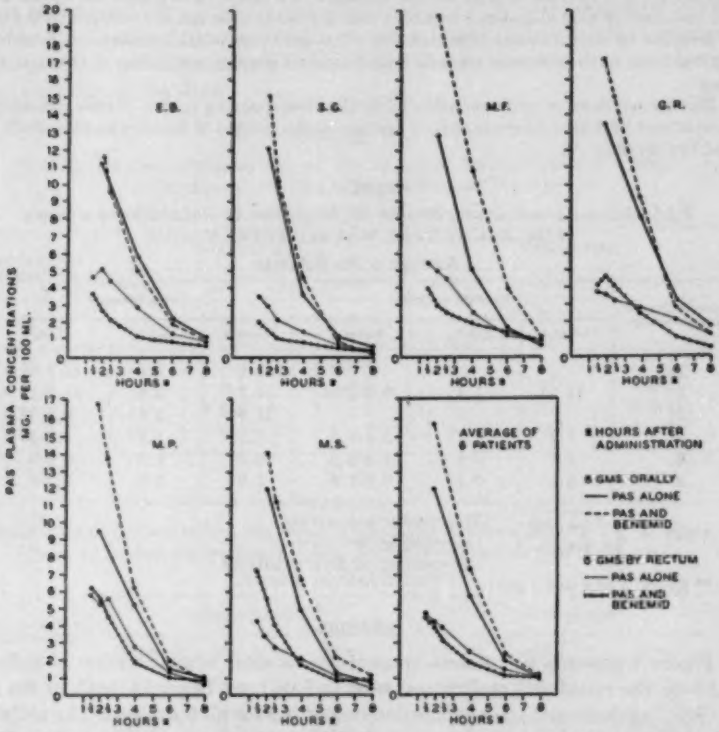

FIG. 1. PAS plasma concentrations after 6 gm. of sodium PAS, with and without Benemid, orally and by rectum.

The plasma concentrations of each subject were determined under the following conditions:

1. Six grams of sodium PAS by mouth, with and without Benemid.
2. Six grams of sodium PAS by rectum, with and without Benemid.
3. Twenty-four grams of sodium PAS in two divided doses, twelve hours apart, by rectum, with and without Benemid.

In every subject, one week was permitted to elapse between each administration of the sodium PAS. The rectal administrations were accomplished by instillation through a cath-

[2] 'Parasal' Sodium is the proprietary name of a twenty per cent solution of sodium para-aminosalicylate obtained from the Panray Corporation of New York City. The authors are indebted to the Panray Corporation for supplying the drug for this study.

eter after the patient had emptied the bladder and a small low-saline enema had been given to cleanse the lower bowel. The foot of the bed was then raised twelve inches, and the patient remained in the recumbent position for two to six hours. The solution of sodium PAS was administered without dilution since it was found in the previous study (1) that a larger volume instilled into the rectum caused distress and a desire to evacuate the bowel. The few instances of mild abdominal cramping that did occur were quickly relieved by 0.4 mg. of atropine by subcutaneous injection. No other gastrointestinal symptoms were noted, nor was there any evidence of mucosal irritation even after an instillation of 12 gm. of the drug.

Blood samples were taken as indicated in the accompanying tables. Plasma concentrations of free PAS were determined by a method similar to that of Bratton and Marshall, as modified by Way (6).

TABLE 1

PAS Plasma Concentrations, Mg. per 100 Ml., After Administration of a Single 6 Gm. Dose by Mouth, With and Without Benemid

Average of Six Subjects

HOURS AFTER PAS ADMINISTRATION	WITHOUT BENEMID			WITH BENEMID		
	Average	S.D.*	Range	Average	S.D.*	Range
1½				17.1	2.4	12.7-18.9
2	11.9	1.4	9.5-13.4	15.7	2.8	11.5-19.9
2½				11.9**	2.3	9.6-14.9
4	5.5	1.4	3.6-7.9	7.1	2.4	4.6-10.8
6	1.8	0.8	1.1-3.2	2.3	1.0	0.9-4.0
8	0.9	0.4	0.5-1.6	1.0	0.4	0.3-1.2

* S.D. $= \sqrt{\sum \frac{d^2}{n-1}}$ S.D.—standard deviation
d—average deviation
n—number of determinations

** Average of five subjects.

RESULTS

Figure 1 presents the plasma concentrations after administration of sodium PAS by the rectal and oral routes, with and without Benemid, both in the individual subjects and the average determinations for all 6 subjects. The individual subjects show considerable variation. With Benemid added to sodium PAS administered by mouth, all but one subject show elevated plasma concentrations at the two-hour level. Patient M. F. demonstrates a sustained elevation up to six hours. In the others, little or no elevation is noted after two hours. When the sodium PAS is given by rectum, the addition of Benemid produces no elevation of the plasma concentration of PAS.

In view of the standard deviations of the average values for each set of determinations as shown in tables 1, 2, and 3, it is apparent that with the exception of the two-hour level after oral administration of sodium PAS, there are no significant increases resulting from the addition of Benemid.

COMMENT

The present investigation was undertaken to determine whether elevation of PAS plasma concentrations could be obtained by the addition of Benemid, par-

ticularly following the rectal administration of sodium PAS. For the purpose of comparison, the combination of Benemid and sodium PAS administered orally was studied in the same subjects. The findings from the study indicate that the addition of Benemid when sodium PAS is given by rectum is of no benefit. The effect of Benemid upon PAS plasma concentrations after oral administration is questionable inasmuch as considerable variation occurs. However, there appears to be evidence of consistent elevation of the plasma concentration two hours after ingestion of the drug.

TABLE 2

PAS Plasma Concentrations, Mg. per 100 Ml., After Administration of a Single 6 Gm. Dose by Rectum, With and Without Benemid

Average of Six Subjects

HOURS AFTER PAS ADMINISTRATION	WITHOUT BENEMID			WITH BENEMID		
	Average	S.D.*	Range	Average	S.D.*	Range
1½	4.6	1.0	3.5-5.9	4.5	1.8	2.1-7.1
2	4.2	1.4	2.9-5.1	3.8	1.7	1.5-5.5
2½	3.8	1.3	2.3-5.6	3.0	1.3	1.1-4.5
4	2.4	0.5	1.5-2.9	1.7	0.6	0.8-2.5
6	1.3	0.5	0.6-2.0	1.0	0.3	0.6-1.6
8	0.8	0.3	0.4-1.1	0.8	0.4	0.4-1.1

* See table 1.

TABLE 3

PAS Plasma Concentrations, Mg. per 100 Ml., After Administration of Two 12 Gm. Doses by Rectum Twelve Hours Apart, With and Without the Use of Benemid

Average of Five Subjects

HOURS AFTER PAS ADMINISTRATION	WITHOUT BENEMID			WITH BENEMID		
	Average	S.D.*	Range	Average	S.D.*	Range
2	11.1	4.3	6.0-16.7	7.9	2.8	3.7-10.3
4	5.3	2.5	2.8-9.5	5.2	3.4	2.1-10.5
8	1.0	0.7	0.4-1.9	1.3	0.6	0.6-2.2
12	0.5	0.6	0.1-1.6	0.4	0.2	0.2-0.7
24	1.0	0.5	0.2-1.3	0.6	0.4	0.0-0.9

* See table 1.

PAS plasma concentrations obtained after the administration of the drug vary considerably from patient to patient. Benemid effect on these plasma concentrations appears to show similar variations. The degree of enhancement obtained from the addition of Benemid to PAS administration does not appear to be sufficient to make it a valuable aid in the treatment of tuberculosis.

SUMMARY

The influence of Benemid on para-aminosalicylic acid plasma concentrations when the PAS was given by mouth and by rectum was investigated.

Although slight variations were observed, there appeared to be no significant increase in the PAS concentrations when Benemid was administered concurrently with the PAS.

SUMARIO

Influjo del Benemid sobre las Concentraciones de Acido Para-Aminosalicílico (PAS) en el Plasma Sanguíneo Después de la Administración Oral y Rectal

Esta investigación versó sobre el influjo del Benemid en las concentraciones plasmáticas del PAS, cuando éste era administrado por las vías bucal y rectal.

Aunque se observaron leves variaciones, no pareció que la adición de "Benemid" introdujera mayores diferencias.

RESUME

(L'influence du Benemid sur les concentrations, dans le plasma, de l'acide para-aminosalicylique (PAS) administré par voie orale ou rectale)

L'influence du Benemid sur les concentrations du PAS dans le plasma quand celui-ci est administré par voie buccale ou rectale a été étudiée.

Bien que de légères variations aient été observées, l'adjonction de "Benemid" n'a déterminé aucune différence appréciable.

Acknowledgments

The authors wish to thank Miss Gloria Fralinger for her invaluable technical assistance.

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THE EFFECT OF THE PROLONGED ADMINISTRATION OF *p*-(Di-*n*-Propylsulfamyl)-BENZOIC ACID (BENEMID) UPON PLASMA CONCENTRATIONS OF PARA-AMINOSALICYLIC ACID[1]

HAROLD L. ISRAEL, FELIX MICK, AND WILLIAM P. BOGER

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INTRODUCTION

The value of para-aminosalicylic acid (PAS) in the treatment of tuberculosis has been firmly established by recently reported controlled studies (1, 2). The administration of this drug daily together with less frequent administration of streptomycin appears to be the most useful chemotherapeutic regimen now available for tuberculosis. This combination has proved so free of serious toxicity and so effective in retarding the emergence of drug-resistant organisms that few tuberculous patients now need to be denied the benefits of chemotherapy. A practical difficulty in the application of this regimen has been the gastrointestinal disturbance resulting from ingestion of PAS. A wide variety of dosage schedules and preparations have been employed in an effort to minimize these troublesome symptoms without loss of therapeutic effect. The administration of PAS in enteric-coated pills gained considerable popularity because of the diminished discomfort; but the greater tolerance to this preparation proved to be merely the result of poor absorption. The daily doses of 20 gm. recommended by Lehmann (3) and other European investigators have not been widely employed in this country because of the anorexia, nausea, vomiting, flatulence, and diarrhea encountered. The schedule adopted in most American studies has been 12 gm. given in four divided doses, but many patients are unable to tolerate even this dosage for prolonged periods.

The development of benzoic acid derivatives which delay the conjugation and excretion of penicillin provided a means of elevating and prolonging the plasma concentrations of this substance. The same benzoic acid derivatives, carinamide and Benemid,[2] have likewise been found to provide elevation and prolongation of PAS plasma concentrations (4, 5). Benemid was reported by Boger and Pitts (5) to effect a twofold to fourfold increase in plasma concentrations of PAS four to eight hours after administration of single 4 gm. doses of PAS. The present study was planned to ascertain the effects of prolonged administration of Benemid and to determine its practical value as an adjuvant to PAS in the treatment of patients under sanatorium conditions.

[1] From the Eagleville Sanatorium, Eagleville, Pennsylvania, and from the Graduate School of Medicine and Section of Infectious Diseases of the School of Medicine, University of Pennsylvania.

[2] Benemid is the Sharp & Dohme proprietary name for *p*-(di-*n*-propylsulfamyl)-benzoic acid; this drug and the effervescent preparation of sodium para-aminosalicylic acid were generously supplied by Sharp & Dohme, Inc.

METHOD OF STUDY

Sixty patients in the Eagleville Sanatorium received Benemid for intervals which ranged from one to five months. Of these patients, 9 were classified as having minimal pulmonary tuberculosis, 20 as having moderately advanced, and 31 as having far advanced pulmonary tuberculosis. A total of 357 trials, each comprising plasma determinations one, three, and five hours after ingestion of a trial dose, was obtained.

Medication was administered with food at 7:30 a. m., noon, 5 p. m., and before retiring at 9 p. m. Blood samples were collected at 1, 3, and 5 p. m., at two- to four-week intervals during the course of therapy. Control determinations of PAS concentrations were obtained before the onset of Benemid administration, and in some instances following discontinuation of Benemid. Plasma concentrations of PAS were determined by the method of Way (6). Benemid was given throughout the study in dosage of 0.5 gm. four times daily. PAS was administered in the form of effervescent tablets, each containing 1.0 gm. of PAS and sodium bicarbonate, which converted the acid to the sodium salt when the tablets were dissolved

TABLE 1

Effect of Benemid upon Mean Plasma PAS Concentrations in 46 Patients on Daily Dosage of 12 Gm. of Effervescent Sodium PAS

	PLASMA PAS CONCENTRATIONS			
	1 hour	3 hours	5 hours	Mean
Benemid	11.55	7.97	4.22	7.91
Control	9.63	5.65	2.44	5.90
Increase	1.92 (19.9%)	2.32 (41.1%)	1.78 (73.0%)	2.01* (34.3%)

* Highly significant difference: P = <0.001.

in water. A small group of patients also received PAS in capsules containing 0.5 gm. of PAS buffered with disodium phosphate. All patients originally received 3 gm. of PAS four times daily, but a majority reduced their dosage during the course of the study to 2 gm. four times daily. A detailed daily record was maintained of toxic manifestations and of consequent reductions in dosage.

RESULTS

Plasma Concentrations of p-Aminosalicylic Acid

Plasma concentrations, attained from ingestion of effervescent sodium PAS alone, corresponded with those reported by investigators (7, 8) employing other preparations. The concentrations were highest at the one-hour point except for a few instances in which higher levels were noted three hours after the test dose. The concentrations five hours after administration of the effervescent tablets were low, and in rare instances measurable quantities were not detected at this interval.

Marked individual variations in plasma levels were noted, and in a few instances higher plasma concentrations were obtained in the control period than

in the period of Benemid administration. Nevertheless, statistical analysis indicates that the mean increase in plasma concentrations of approximately 30 per cent (tables 1 and 2) as the result of Benemid is significant. An enhancement of similar proportions was observed in patients who received buffered sodium PAS capsules (table 3).

Benemid thus exerts a consistent action in increasing plasma PAS levels but the effect is not as great as that observed in the original acute experiments. To determine whether the lesser enhancement was due to prolonged administration, the plasma concentrations obtained in the first month of Benemid therapy were

TABLE 2

Effect of Benemid upon Mean Plasma PAS Concentrations in 14 Patients on Daily Dosage of 8 Gm. of Effervescent Sodium PAS

	PLASMA PAS CONCENTRATIONS			
	1 hour	3 hours	5 hours	Mean
Benemid	7.95	5.24	2.38	5.19
Control	6.62	3.87	1.60	4.03
Increase	1.33 (20.1%)	1.37 (35.4%)	0.78 (48.8%)	1.16* (28.8%)

* Significant difference: P ≅ 0.01.

TABLE 3

Effect of Benemid upon Mean Plasma PAS Concentrations in 7 Patients on Daily Dosage of 8 Gm. of Sodium PAS Capsules

	PLASMA PAS CONCENTRATIONS			
	1 hour	3 hours	5 hours	Mean
Benemid	8.29	6.37	5.21	6.62
Control	5.79	4.67	3.90	4.79
Increase	2.50 (43.2%)	1.70 (36.4%)	1.31 (33.7%)	1.83* (38.2%)

* Highly significant: P = <0.01.

compared with those obtained in the second and third months and in the fourth and fifth months. No significant differences were observed in the effect of Benemid in these three periods (table 4).

Observations on Emergence of Streptomycin-Insusceptible Tubercle Bacilli

An attempt was made to ascertain whether the addition of Benemid to combined streptomycin-PAS regimens would, by increasing PAS concentrations, more effectively retard the emergence of streptomycin-resistant organisms. The administration of streptomycin in daily dosage of 1.0 gm. for a total of forty-two

days has been shown to result in drug resistance in 25 per cent of patients with cultures positive for *M. tuberculosis* on completion of the course (9). The addition of PAS has been shown to reduce the frequency of the phenomenon of emergence of streptomycin-resistant tubercle bacilli (10). A comparison was instituted of the relative frequency of emergence of streptomycin-resistant microorganisms in patients given forty-two-day courses of streptomycin and PAS, with and without Benemid. The demonstration of the striking success of intermittent streptomycin administration in prevention of resistance and the adoption of this regimen as a routine treatment made it necessary to terminate this study before an adequate number of patients could be treated. Five of a group of 6 patients who did not receive Benemid had repeated cultures negative for tubercle bacilli after treatment. Cultures of *M. tuberculosis* obtained from the remaining patient retained susceptibility to streptomycin. Thirteen patients received Benemid; post-treatment cultures were positive for *M. tuberculosis* in 7, and in 4 the microorganisms were drug-susceptible. These observations are too few to provide evidence concerning the emergence of drug resistance and

TABLE 4

Effect of Prolonged Administration of Benemid upon Plasma PAS Concentrations

PAS DOSAGE	DURATION OF BENEMID	NUMBER OF TRIALS	MEAN PLASMA PAS CONCENTRATIONS
2 gm. four times daily	0–1 month	32	5.1
	1–3 months	41	5.3
	3–5 months	20	5.7
3 gm. four times daily	0–1 month	71	8.0
	1–3 months	43	7.4
	3–5 months	17	6.3

are chiefly remarkable in showing the frequency (58 per cent) with which combined streptomycin-PAS therapy succeeded in achieving reversal of infectiousness.

Toxicity

Benemid: No toxic symptoms attributable to Benemid were observed.

Para-aminosalicylic acid: No instances of toxicity to PAS were observed in the 60 patients under study except for gastrointestinal symptoms. Anorexia, nausea, flatulence, vomiting, and diarrhea were extremely common, however, and only 7 patients were able to go through the course of treatment without a single interruption due to gastrointestinal distress.

In an attempt to evaluate more precisely the importance of gastrointestinal symptoms, a record was made of all interruptions of therapy or reductions in dosage. This appeared to provide an objective index to the severity of the gastrointestinal symptoms. The tolerance of patients to the various dosage schemes employed has been graded as *poor*, *fair*, and *good* according to the length of

intervals during which dosage was tolerated without reduction. The following definitions were employed in classifying the tolerance of 52 patients with reliable records: *good:* tolerance for thirty or more consecutive days; *fair:* tolerance for intervals of fifteen to thirty days, with few interruptions; *poor:* tolerance for intervals of less than fifteen days, with many interruptions. It should be recognized that patients classified as having good tolerance were by no means free of gastrointestinal symptoms, but merely exhibited greater stoicism or determination.

Of the total of 52 patients started on a regimen of 12 gm. of PAS plus Benemid, 37 (71.1 per cent) reduced their dosage to 8 gm. daily. On the other hand, only 3 (5.8 per cent) of the entire group exhibited poor tolerance to the regimen of 8 gm. daily and Benemid.

Relation of tolerance to body weight: An analysis has been made of the relationship of tolerance to the weight of the patient, a factor not usually taken into consideration in the administration of PAS. Tolerance to 12 gm. of PAS plus Benemid was classified as good in 11, fair in 3, and poor in 5 patients weighing 150 pounds or more; tolerance among patients weighing less than 150 pounds was classified as good in 6, fair in 4, and poor in 18 instances. A schedule of 12 gm. of PAS and Benemid would therefore appear worthy of trial in patients weighing 150 pounds or more but to have little likelihood of success in smaller or thinner patients.

It is not clearly established whether the gastrointestinal symptoms accompanying PAS administration are due to local irritation or represent a central effect related to the blood concentration. It is noteworthy that despite many investigations the same question remains concerning the symptoms produced by the large doses of salicylates employed in treatment of rheumatic fever. It has been established that when PAS is given intravenously (11) or by enema (12) high blood concentrations are better tolerated than comparable concentrations obtained by ingestion. However, patients in this study, whether taking 8 gm. or 12 gm., appeared to have some increase in symptoms after adding Benemid to their schedule. This suggested that symptoms might be due to the higher plasma PAS levels achieved, since there is no evidence for gastrointestinal irritation due to the Benemid itself. Six patients given Benemid alone noted no distress; and 2 gm. of Benemid together with 8 gm. of PAS were well tolerated while the same dose of Benemid with 12 gm. of PAS was not. An effort was therefore made to determine whether tolerance to PAS could be correlated with the plasma concentrations observed. No correlation could be established.

DISCUSSION

The action of Benemid in increasing plasma PAS concentrations is confirmed by the present study, but the degree of enhancement is smaller than that described in the original report on observations made after ingestion of single test doses of PAS.The earlier trials were made after administration of larger doses of PAS, i.e., 4 gm., than were used in the present study; and it should be noted that the fourfold enhancement reported originally was observed only six to eight

hours after ingestion, at a time when the plasma concentrations of PAS were small and not measurable with great precision.

Although the increase in PAS concentrations effected by Benemid administration was small, this drug may prove of clinical importance. It was considered initially that by employing Benemid in conjunction with 12 gm. of PAS, the dosage that has been generally adopted in this country, markedly enhanced blood levels and presumably a greater therapeutic effect could be obtained without concomitant increase in toxicity. Benemid appears to have limited practical value for this purpose; only a small minority of patients was able to tolerate the 12 gm. PAS regimen for prolonged periods. On the other hand, 8 gm. of PAS daily, together with Benemid, was well tolerated by nearly all patients and provided plasma PAS concentrations almost as high as those obtained by use of 12 gm. of PAS alone, with less gastrointestinal distress.

The dosage of PAS and the plasma PAS concentrations required for maximum therapeutic effect have not been established. A preliminary report by Warner (13) suggests that the 8 gm. dose is less effective than the 12 gm. schedule in retarding the emergence of streptomycin-resistant microorganisms. There is need for additional study to determine whether there is sufficient difference in therapeutic effect between these two regimens to justify continued employment of the higher dosage which is so poorly tolerated by many patients. If the higher blood concentrations obtained with the larger dose are associated with a greater therapeutic effect and slower emergence of drug-resistant microorganisms, then Benemid may prove a valuable supplement to the 8 gm. daily dosage of PAS. It should be emphasized that in any comparative study of the bacteriologic and clinical effects of various PAS dosages, it is essential that plasma PAS concentrations be actually determined since they vary widely with different preparations of PAS.

SUMMARY

Sixty patients with pulmonary tuberculosis have been given para-aminosalicylic acid and Benemid orally for periods which ranged from one to five months, and the plasma PAS concentrations were repeatedly measured. A mean increase of approximately 30 per cent in plasma PAS concentrations was observed after addition of Benemid.

No toxic symptoms attributable to Benemid were observed. Prolonged administration of 12 gm. of PAS plus Benemid resulted in marked gastrointestinal symptoms while tolerance to 8 gm. of PAS plus Benemid was good. Benemid has little clinical promise as a supplement to high doses of PAS, but appears valuable as a means of increasing plasma PAS concentrations in patients who can only tolerate small doses of PAS.

SUMARIO

Estudio Prolongado del Efecto de Benemid sobre los Tenores de PAS en el Plasma

A 60 tuberculosos pulmonares se les administraron PAS y Benemid por vía oral durante períodos de tiempo que llegaron hasta cinco meses, midiéndose

repetidamente las cifras de PAS en el plasma. El empleo de Benemid dió por resultado un aumento de 30 por ciento en las concentraciones plasmáticas de PAS.

No se observaron síntomas tóxicos imputables a Benemid. La administración prolongada de 12 gm. de PAS más Benemid fué seguida de pronunciados síntomas gastrointestinales, mientras que fué buena la tolerancia a 8 gm. de PAS más Benemid. El Benemid ofrece pocas posibilidades clínicas como complemento de dosis altas de PAS, pero parece valioso como medio de acrecentar las concentraciones plasmáticas de PAS en enfermos que no pueden tolerar más que pequeñas dosis de esta droga.

RESUME

Une étude prolongée de l'action du Benemid sur la concentration du PAS dans le plasma

Soixante malades atteints de tuberculose pulmonaire ont reçu du Benemid et du PAS par voie buccale pendant des périodes pouvant atteindre cinq mois au cours desquelles la concentration sanguine du PAS était constamment mesurée. L'usage du Benemid a permis d'obtenir une augmentation de 30% de la concentration du Benemid dans le plasma.

Aucun signe de toxicité imputable au Benemid n'a été observé. L'administration prolongée de 12 g. de PAS avec en plus du Benemid a été accompagnée de troubles gastro-intestinaux marqués tandis que 8 g. de PAS plus le Benemid ont étés bien tolérés. Le Benemid ne semble pas offrir de perspectives favorables en clinique comme adjuvant à de hautes doses de PAS, mais il paraît devoir être utile comme moyen permettant d'augmenter la concentration du PAS dans le sérum chez les malades qui ne tolèrent que de petites doses de PAS.

Acknowledgment

We wish to thank Elizabeth Kane, R.N., of the Eagleville Sanatorium laboratory for securing specimens, and Mr. Joseph Ciminera of Sharp & Dohme, Inc., for statistical analyses.

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THE IMPORTANCE OF RESPIRATORY DISEASES[1, 2]

ALEXANDER D. LANGMUIR

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In speaking to you I am pinch-hitting for Dr. Gaylord W. Anderson, Chairman of the American Trudeau Society's Committee on Respiratory Infectious Diseases. The title of my speech, "The Importance of Respiratory Diseases," has been provided for me either by Doctor Anderson or by Doctor Perkins. Unfortunately, neither of these estimable gentlemen has provided me with a manuscript. Nevertheless, I welcome the opportunity to speak on this subject because I believe in it. I speak for myself and not for Doctor Anderson, Doctor Perkins, the Committee, or the Public Health Service.

I propose to examine some of the general epidemiologic principles which have led to our successful control of certain infectious diseases. I shall attempt to analyze the prospects of substantial improvement in the control of the many respiratory diseases which still plague us. May I quote from the book, *Plague on Us*, by Mr. Geddes Smith.

> Great and small, the respiratory infections are indubitably unfinished business. These common ailments form a nosological jungle in which bacteria and viruses roam at will, despoiling the human race and defying both classification and control. Symptoms overlap and no one knows how many different diseases lurk behind them. For some of them the doctor can do little. The epidemiologist who hacks his way into this mess courts frustration. The statistician has to content himself with omnibus calculations. The plain citizen talks glibly of grippe or flu, gulps or sniffs his favorite panacea, and, without any clear notion of what is happening to him, hopes for the best.

Had I the chance of choosing my title for this speech, perhaps I should have chosen the subject, "Courting Frustration in a Nosological Jungle," or, as you will hear, a more appropriate title would be "The Possibilities of Eradicating Respiratory Diseases."

In considering my broad subject, I would like to start by challenging a widely held belief that has inhibited, and even frustrated, those of us who have chosen the public health profession. I refer to the proposition that the communicable diseases are now conquered and, therefore, need little further concerted effort.

Many of my good friends tell me I am wasting my efforts by working in a dying field. They say that the future lies in the noninfectious, the metabolic, and the chronic debilitating diseases of older ages.

I challenge this proposition. While I will not deny the importance of noninfectious diseases, I maintain that much remains to be done in the communicable

[1] From the Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Georgia.

[2] Presented before the Medical and Public Health Sections, as part of the *Joint Meeting* at the 47th annual meeting of the National Tuberculosis Association, Cincinnati, Ohio, on May 17, 1951.

diseases. We heard from Doctor Dubos last night that he believes in further research in microbiology. I need not argue to this audience that tuberculosis is still a problem in spite of Doctor Smith's toboggan-slide curves of tuberculosis mortality. I firmly believe that in the field of the infectious diseases there is a happy hunting ground for major discoveries and contributions to the welfare of mankind that will equal, if not surpass, for some time to come those to be made in the field of the degenerative diseases.

Most of the infectious diseases may be classified into three broad groups as follows:

(*1*) The enteric infections
(*2*) The arthropod-borne infections
(*3*) The respiratory infections.

In Western civilization, progress in the control of the first two of these three groups of diseases has been impressive. We have practically eliminated typhoid fever and the dysenteries. Yellow fever and dengue are gone from this country and Northern Europe. Typhus and plague are now trivial problems. Perhaps the most impressive of all is the story of malaria. This disease was heavily endemic and seemingly permanent in large areas of the South in the mid nineteen thirties but now has disappeared as a naturally spread disease.

In contrast, our control of the respiratory group is *un*impressive. Smallpox is our only total success. We can take considerable pride in the record of diphtheria. We have only begun to apply our knowledge effectively against whooping cough. The treatment of streptococcal infections, bacterial pneumonia, meningitis, and the bacterial complications of measles and influenza has been materially benefited by the specific antimicrobial therapies; but the beginning is yet to be made in their effective control or elimination. Poliomyelitis, infectious hepatitis, mumps, chicken pox, the common cold, and that group often termed the "undifferentiated respiratory diseases" continue amongst us unabated. "Indubitably," to quote Mr. Smith again, "The respiratory diseases are unfinished business."

Why have we been so successful with the enteric and arthropod-borne diseases and yet have so largely failed with the respiratory infections? The answer is clear. The former two groups of infections depend for their survival either on gross fecal contamination of the environment or on the close association of insects or rats with the human population. These basic conditions for survival have been eliminated by both conscious public health effort and as a beneficent concomitant of an advancing standard of living.

The reasons for our failure with the respiratory diseases are equally understandable. These infections depend for their survival on direct person-to-person transmission which cannot readily be attacked by the broad community approach of environmental sanitation. In the few respiratory diseases which we have successfully controlled, we have depended on immunization. While all of us, even health officers, have a personal aversion to needles, I believe that the principle of immunization provides the most promising basis for future advance.

What are the prospects? I believe they are very real. Let us first apply what we now know. There is little excuse for most of the 9,600 reported cases and 634

deaths from diphtheria in 1948 (the last year for which national mortality figures are available) or for the 74,000 reported cases and 1,100 deaths from whooping cough. These could be materially reduced and possibly eliminated by the effective application of existing knowledge. This problem is essentially one of health education of the medical and public health profession and the general public. This organization has a long and brilliant record in these techniques.

Next, let us look at other respiratory diseases for which a generally accepted immunizing agent is not now available. The viruses of influenza and mumps can be grown in the embryonated hen's egg. This means that antigen can be made available in almost limitless quantities. Thus, the major stumbling block to the preparation of a vaccine has been licked. While I do not wish to minimize the still substantial developmental problems remaining to be solved before a practicable, safe, and effective immunizing agent for generalized use in the population can be available for these two diseases, it seems wholly reasonable that this objective can be achieved for both in the near future. The isolation of the viruses of measles, infectious hepatitis, and the common cold has been reported. This means the first step toward a vaccine has been taken, but whether or not adequate amounts of antigen can be produced is still problematic.

A recent development announced by Dr. Jonas Salk in Atlantic City just two weeks ago has direct bearing on our problem of immunization. In my judgment it constitutes a very major advance. By starting with the pioneer work of Dr. Jules Freund, who opened up the field of the use of adjuvants to enhance antibody response, Doctor Salk has found that, by selecting a simple mineral oil of low viscosity and the right detergent or emulsifying agent, he can obtain in both monkeys and man high and sustained influenza antibody titers with but a single injection. Furthermore, the material is essentially reaction free, and the total antigen required is fractional compared with the amount formerly used in influenza vaccines.

This discovery means that many stumbling blocks to the control of respiratory diseases have been removed. One of these is that the small amount of antigen required indicates that multiple types and substrains of influenza viruses may be included in one inoculation, thereby giving a much broader antibody response than has been attainable heretofore. While still new and different antigenic strains of influenza virus may appear in the population, it would seem that we are much nearer to the development of a practical influenza vaccine than we were prior to Doctor Salk's development.

Moreover, this work quite adequately disposes of two commonly accepted fallacies. The first is that we cannot expect to achieve by artificial means a greater immunity than is created by naturally acquired infection. Doctor Salk has clearly produced antibody responses that regularly exceed the natural response in influenza. While I freely admit that the titer of antibody in the circulating blood may not be a direct measure of immunity, there is certainly an established relation in influenza and in a number of other infectious diseases. I believe that we can look to the future field trials with the new influenza vaccines with considerable enthusiasm.

The second fallacy is the assumption that to achieve substantial immunity

two or more properly spaced doses of immunizing agent are necessary; the first, to condition the virgin susceptible to an initial response; and the second to act as a booster dose to bring out the recall phenomenon and lead to higher and more sustained titers. With Doctor Salk's preparation antibody responses to a single dose regularly exceed the titers achieved after multiple doses of saline prepared vaccine.

Not only does this discovery lend solid prospect to great simplification of existing immunizing procedures for a variety of agents, but it also offers real hope of achieving useful and effective vaccines for new agents that now are intrinsically weaker antigens or more difficult to prepare in adequate concentrations. It may well be possible to give a wide variety of antigens in one dose. We should begin to think seriously in terms of a dozen or more antigens in one reaction-free dose. This, veritably, would be a magic bullet.

May I draw the conclusion that the prospects for major advances in the future control of respiratory diseases are bright; but we still have a long way to go, both in applying what we now know and in making new discoveries.

What is the theoretical limit we can shoot for? I think it is clear, but to discuss this I must challenge another commonly held belief, even a fetish in many circles. It is the proposition that *eradication* of an infectious disease is not an attainable or practicable goal. If one will accept Dr. Justin M. Andrew's definition of the concept, namely, area-eradication or the elimination of the natural spread of the disease in a large contiguous area, such as the United States, I maintain that eradication is a reasonable and attainable objective for a number of diseases. It was achieved for yellow fever and dengue decades ago, and within the past decade even for malaria.

In the past five years the Communicable Disease Center has made an intensive search for evidence of the occurrence of mosquito-transmitted malaria in this country. While a small number of single verified cases has been uncovered, they have almost all been evident relapses, introduced cases, or transfusion malarias. A very few are unexplained, but not a single instance of two or more cases occurring in epidemiological relation has been discovered. This constitutes area-eradication according to Doctor Andrew's definition.

These achievements are not limited to the arthropod-borne infections. Since 1948 less than 100 cases of smallpox have been reported each year from the entire nation. These few cases are not concentrated along the Mexican border or the port cities where occasional introductions may well occur; and, therefore, it is a reasonable epidemiologic conclusion that they are erroneous diagnoses. The last outbreak of smallpox in this country that I have been able to find occurred along the Mexican border in 1949. While to the purists we cannot claim eradication, we all know that this has been achieved for all practical purposes.

I maintain that to argue, as some do, that no infection can be considered as eradicated from an area because it may be accidentally introduced from outside shrouds the significant fact that the disease has been successfully eliminated. This rigid semantic position inhibits us from declaring our logical objectives and makes us complacent with partial success.

Let us examine the epidemiologic basis for the disappearance of smallpox. This leads to the subject of epidemic theory developed first by Farr, Hamer, Brownlee, and Soper in England and extended in this country by Frost, Reed, and Wilson. It is axiomatic that, for the survival of any disease which is an obligate parasite of man, one infected individual must give rise on the average to one new infected individual. This ratio, of course, may vary. If at one particular time the circumstances are such that one case gives rise to more than one case in the next generation, the incidence rises. As the epidemic progresses, however, recovered cases become immune and the susceptibles become depleted to the point where new cases no longer give rise to an equal number of subsequent cases. Then, the incidence falls and the epidemic subsides. For respiratory diseases such as measles, influenza, and many others the epidemic terminates long before the susceptibles are exhausted. Another epidemic does not recur until the susceptibles are replenished by the addition of new births as in measles or by waning immunity as in influenza.

The agents of this group of diseases may be said to have achieved a successful biologic balance with the human race. Their incidence fluctuates over short time intervals; but, averaged over longer time spans, it has been remarkably stable.

The epidemic theorists have written a simple equation that accounts for this waxing and waning of incidence of such diseases as measles. The incidence rate may be expressed as a function of only two factors:

(*1*) The proportion of susceptibles in the population
(*2*) The contact rate.

The former is a simple concept which can be measured in at least approximate terms for several diseases. The latter is a composite of many variables including the ease of transmission of the particular infection from person to person and the frequency with which people come in contact with each other in a given time period. Thus, the contact rate is a statistical parameter determined by the biologic characteristics of the host-parasite relationship and the standard of living. For diseases such as measles and mumps, in which the host-parasite relationship has been stable for centuries, the important factor determining the incidence rate is the proportion of susceptibles in the population.

For each contagious disease there is a certain threshold of susceptibles which, if it is exceeded, leads to the occurrence of a rising incidence or an epidemic; and, conversely, if the proportion of susceptibles is less than the threshold, no epidemics can occur. The characteristic two- to three-year periodicity of measles, which occurs so regularly in many of our cities, can be adequately accounted for essentially in these simple terms.

On the basis of this theory, the eradication of respiratory disease follows logically. All that is necessary is to maintain the threshold by artificial means, such as immunization, well below that which is necessary for one case to give rise to one subsequent case. If this reduction in susceptibles is maintained effectively and generally over a large area, such as a whole nation, and particularly in pockets of the population where the contact rate is especially high, the disease

must steadily and progressively disappear. With a truly effective program of immunization this disappearance should be rapid, a matter of only a few years.

It should be emphasized that the conditions necessary for the disappearance of such an infection do not, by any means, require that the total population be immunized. Probably a 50 per cent reduction in the proportion of susceptibles, if well distributed, would be sufficient.

This is exactly what has happened with smallpox. Although it is now 150 years since Jenner's discovery, we substantially achieved this goal many years ago. We have arrived there in spite of the fact that vaccination is by no means universal and the immunity conferred by vaccination is neither absolute nor permanent. Vaccination is a sufficiently widespread practice, particularly as a requirement for admission to school, that the threshold of susceptibility to the disease has been constantly maintained at a level where the disease was forced to disappear as an endemic infection.

Two additional factors are important in this achievement. The *first* is the rigidly enforced requirement that all travelers, both immigrants and tourists, must have a recent successful vaccination before entering the country. This materially reduces the chance of introduction. The *second* factor is the popular clamor and demand for vaccination whenever a case of smallpox is reported. The *primary* factor, however, is the routine vaccination of our school children which keeps the threshold at a safe, low level. If we fail to maintain this practice, we expose ourselves to danger.

I believe these principles are epidemiologically sound and generally applicable to any acute infectious disease which is an obligate parasite of the human race and for which acquired immunity is the factor controlling incidence. They certainly apply to measles, mumps, and chickenpox in which a high proportion of the total infections are clinical cases and immunity is long lasting. I can see no theoretical reason why they should not apply to diseases such as diphtheria, whooping cough, and poliomyelitis in which a higher proportion of infections is inapparent. The streptococcal, pneumococcal, and meningococcal infections and influenza present special problems because of the multiplicity of immunologically distinct types. These may raise practical problems in the development of specific vaccines but not basic theoretical objections of an epidemiologic nature. The basic requirement is an effective immunizing agent.

In summary, then, the necessary conditions for the area-eradication of the common respiratory and contagious diseases are:

(*1*) The reduction of the threshold of susceptibles to a level where one case gives rise, on the average, to less than one case in the next generation. This implies rather widespread and continuing practice of immunization and the availability of a safe, effective, and practical immunizing agent. It does not imply universal immunity.

(*2*) The maintenance of effective requirements of immunization of immigrants and tourists entering the country to prevent introduction of the disease.

(*3*) A constant and vigilant epidemiologic surveillance, supported by an informed and cooperative public, to stamp out by intensified immunization any accidental introduction that may occur.

The respiratory diseases are unfinished business. Etiologic discoveries, particularly the cultivation of specific agents in the chick embryo, and the simplification and enhancement of immunizing procedures give promise of new achievements in the near future. The area-eradication of respiratory diseases is the ultimate goal which should be theoretically attainable for a number of respiratory infections. When this is achieved, the noninfectious, chronic, debilitating diseases will justly command the primary attention of the epidemiologists.

Correspondence

TUBERCULIN-NEGATIVE TUBERCULOSIS

To the Editors of The American Review of Tuberculosis:

I feel compelled to write you relative to the first article in the May 1951 issue of THE AMERICAN REVIEW OF TUBERCULOSIS entitled "Tuberculin-Negative Tuberculosis" by Willi Mascher. I think it particularly important to call the attention of tuberculosis physicians to certain statements made in this article which seem to me to be unproved and which certainly give erroneous ideas about the usefulness of the tuberculin test.

In the first place, a critical review of Doctor Mascher's cases shows that he presents 5 cases of probable tuberculosis with negative tuberculin, 2 cases of questionable tuberculosis, and 3 in which the diagnosis is extremely questionable. On the basis of these 10 patients, Doctor Mascher makes very extensive generalizations regarding the usefulness and limitations of the tuberculin test.

Doctor Mascher reports finding 8 cases with negative reactions in a survey of some 178,000 persons. Even if one does not question his diagnosis of tuberculosis in these cases, the fact remains that only 5 persons in 1,000 who had tuberculosis showed a negative tuberculin. In addition, he presents evidence of 3 cases found in his sanatorium work in the past four years. Both of these statements, it seemed to me, are of extreme importance; namely, that he was able to find *only* 3 cases which he could question in four years of study at his sanatorium and that he found *only* 8 cases which he questioned in his survey of more than 178,000 inhabitants of his district. Thus, from the author's own figure one must conclude that tuberculin-negative tuberculosis is extremely rare. If only one or two cases in a thousand cases of tuberculosis show a negative tuberculin, it would seem that the tuberculin test would still be very useful and effective for the diagnosis of tuberculosis.

It is difficult to imagine any test which does not have at least an occasional error due to biological variation or technical difficulties. On the other hand, if the error is only one or two cases in a thousand cases of tuberculosis and if we are using the tuberculin test to assist us in the diagnosis of tuberculosis among general population groups in which surveys indicate that only one per cent or less have tuberculosis, it is easy to see that the diagnostic error is something which should cause little concern clinically. I would like, therefore, to take exception to the statement made on page 508 that "For practical clinical purposes it is particularly important to remember that *a negative tuberculin reaction in a case of suspected pulmonary disease does not exclude tuberculosis* even in a patient who is in a good general condition and is free from intercurrent nontuberculous infection." As a matter of fact, it would appear that, if the negative were omitted from that statement, it would come closer to expressing the facts. In other words, a negative tuberculin reaction does for all practical clinical purposes exclude the presence of tuberculosis. A considerable portion of the paper is devoted to discussion of reverters in tuberculin and references made to certain studies which have shown a variety of reversion rates. However, analysis of these studies by the size of dosage of tuberculin used indicates that there is a distinct quantitative relationship in the reversion rate. This is particularly brought out by the study of Dahlstrom which is referred to. If Dahlstrom's figures are analyzed on the basis of the first and second doses of tuberculin, it is found, among reactors to the first dose of tuberculin who were followed over an average period of nine years, that the reversion rate was only about 2 per cent; whereas among the same group who reacted only to the

second dose of tuberculin the reversion rate was something like 60 per cent. We have had similar experiences in certain unreported studies.

Perhaps this whole paper headlights the importance of the quantitative consideration of the tuberculin test. It is evident from the second paragraph that the author considers that tuberculin is specific in any dosage employed. This consideration appears to be based upon his review of the literature and has nothing to do with his experimental findings as reported later in the paper. It has been shown in numerous studies by ourselves and others that tuberculin in large doses may induce nonspecific reactions and that persons with tuberculosis are, in general, extremely hypersensitive to tuberculin. These studies have shown that a dose of somewhere in the neighborhood of first testing dose or a little larger is satisfactory for selecting persons who are sensitive to tuberculin with reasonable accuracy. The use of larger and larger doses of tuberculin, as was done in past studies, serves simply to obscure the problem due to the presence of nonspecific reactions to these large doses. It seems that the prominent place given this article in THE AMERICAN REVIEW OF TUBERCULOSIS might lead certain workers to consider that tuberculin-negative tuberculosis is of frequent occurrence. This is not the case as is shown by review of the author's figures. At any rate, it would appear wise to call the attention of tuberculosis physicians to the large mass of evidence indicating, first, that tuberculin is specific when employed in relatively low doses only; and, second, that persons with tuberculosis, with very few exceptions, react to tuberculin. It seems to me that Doctor Mascher's article serves only to emphasize these two points rather than call into question the clinical value of the tuberculin test.

Finally, it might be well to speculate on the immunological implication which arises from Doctor Mascher's article. He has shown as well as others before him that an occasional case of proved tuberculosis fails to react to tuberculin in the absence of any determinable depressing factors. That this is possible no thoughtful biologist would deny. Even though, as stated above, it does not occur frequently, it suggests that those persons who do not react as the great majority of patients are in some way unusual, either in their infecting organism or in their response to this organism. The failure to respond to vaccination with either BCG or their own organisms suggests that the difficulty lies in some abnormality or deficiency in their immunological response to infection. It would seem that further studies of these "anergic" infected persons by the use of spreading tests with dyes or the effect of repeated vaccination, possibly with the addition of adjuvants, and finally the prognostic implications of this anergy to the patients themselves would yield most interesting and useful results to the clinician.

Much of this last paragraph has been stimulated by correspondence with the Managing Editor of the REVIEW, Dr. Walsh McDermott, regarding this most interesting paper.

I/C Investigations Section
OFFICE OF MIDWESTERN CDC SERVICES
3900 EATON STREET
KANSAS CITY, KANSAS

Respectfully,
MICHAEL L. FURCOLOW

July 19, 1951

To the Editors of The American Review of Tuberculosis:

I am very thankful for your kindness in offering me this opportunity of writing some lines in answer to Doctor Furcolow's most interesting letter.

First of all, I should like to express my astonishment at the fact that any reader could find in my article, "Tuberculin-Negative Tuberculosis", any doubt in the specificity of

the tuberculin reaction itself or in the enormous value of this test in epidemiologic studies or in clinical work. I should like to assure Doctor Furcolow, and any other reader of my paper to whom I might have given "erroneous ideas about the usefulness of the tuberculin test," that the value of this test for me is a truism which no longer needs to be discussed.

But, trying to live up to "a thoughtful biologist" (as Doctor Furcolow puts it) in the first part of my paper, lining up the background of my investigations, I tried to find out the limits regarding the conclusions one is allowed to draw from this test *when it turns out negatively*. In my paper, as I was dealing with cases where there was no reaction at all, I omitted all discussion about the possibility of nonspecific reactions. Nevertheless, I should like in this place to declare that I agree perfectly with Doctor Furcolow in his warnings against nonspecific reactions, especially when higher doses are used. Here also I might add that even the number of positive reactions, at least in the Scandinavian countries, is highly influenced nowadays by the great and steadily increasing number of BCG-vaccinated persons, a fact which makes the epidemiologic interpretation of tuberculin surveys rather difficult. In the future we will have to reckon with an unknown number of tuberculin-negative persons on account of "reversion" (see pages 504 and 505 of the paper) and tuberculin-positive persons on account of BCG vaccination. This will not in any way make tuberculin testing less valuable or less necessary but it surely will make it still more interesting!

However, the very purpose of the study reported in the May number of the REVIEW was to investigate cases with active tuberculosis and negative tuberculin reaction. This is not an epidemiological question and not a question of statistics. I think that a study of this kind might be of some value even if these cases admittedly are not common. From the clinician's point of view, it must be of the greatest importance not to miss a single case on behalf of falsely drawn conclusions. Statistically, it may be well allowed to state that "negative Mantoux test excludes active tuberculosis." Nevertheless, from the standpoint of the tuberculosis physician in his diagnostic work, this statement is not true in every case. There may be many or few of that kind but they have to be correctly diagnosed. In 1943 Hedvall had 7 cases (all with *M. tuberculosis*) and there are many other authors who have reported single cases. I myself collected the reported 11 cases. The reservations made in Doctor Furcolow's "critical review" of my cases are really a citation of my own categories, with the difference that I interpreted the cases as "6 cases with unequivocal tuberculosis, 2 cases with almost certain tuberculosis, and 3 cases in which the presence of active tuberculosis is considered to be highly probable" (page 515). Well, even if one or another of these cases may be questioned, there remains the fact stated "that a negative tuberculin reaction *in a case of suspected pulmonary disease* does not exclude tuberculosis (page 508). I believe that it is worth while to state this fact (which I am not the first to state) as it is not as widely known as it ought to be and also on behalf of its clinical relevance.

Regarding the frequency of these cases in my material, I quite agree with Doctor Furcolow that they are not common. On the other hand, I never "reported finding 8 cases which were tuberculin negative in a survey of some 178,000 persons." The fact is (page 507) that, of 5,623 tested persons *with suspicious roentgenological findings*, 196 turned out to be tuberculin negative. Among these, I found 8 cases with active tuberculosis. Of course, it is quite correct to say, as Doctor Furcolow does, that *only* 5 persons in 1,000 who had tuberculosis showed a negative tuberculin. But it is just as correct to state that 4 in 100 persons with suspicious roentgenologic findings showed a negative tuberculin in spite of the fact that they suffered from active tuberculosis.

Moreover, in my opinion these cases not only have clinical relevance but they also are of the greatest scientific interest. It is really a very bewildering and remarkable fact that

there are (few or many) individuals who do not react to tuberculin in the usual way in spite of the fact that they expectorate *M. tuberculosis*, and some even show cavernous disease. As long as possible I want to believe that the negative cutaneous test does not mean lack of hypersensitivity of the entire host. Unfortunately, I do not know any other way that would be safe for the patients to test their total hypersensitivity. I believe that I have shown in my paper that it is not the general reactive capacity of the skin which causes this negative reaction (page 516) nor is it the *M. tuberculosis* found in these patients, for the bacilli were apparently in no way different from other strains (page 517). I am now continuing the reported investigations with BCG vaccination and cutaneous vaccination with the patients' own or other strains and am also continuing with a study of Jadasson's hypothetic "anticutins." In collaboration with the bacteriological and the chemical laboratories of the University of Upsala, I am investigating the production of antibodies and the chemical constitution of the serum of these patients. I hope later on to be able to publish some more data on these highly interesting but very difficult questions.

Finally, I would like to say that I am very thankful for this opportunity given by the courtesy of the editors of THE AMERICAN REVIEW OF TUBERCULOSIS to state that from an epidemiological point of view and with regard to the value of the tuberculin reaction as a test there is no difference between Doctor Furcolow's viewpoint and my own.

SÖDERMANLANDS LÄNS SANATORIUM
LÖT, MALMBY
SWEDEN

Yours very sincerely,
WILLI MASCHER

July 29, 1951

REQUEST FOR DATA ON EFFECTS OF CORTISONE AND ACTH ON TUBERCULOSIS IN HUMANS

To the Editors of The American Review of Tuberculosis:

The Committee on Therapy of the American Trudeau Society wishes to ask the cooperation of the medical profession in a survey of the possible deleterious effects of cortisone and ACTH on tuberculous infection in man. Experimental work has indicated this possibility, and scattered clinical experience has suggested that both compounds may have a harmful effect in human tuberculosis.

The American Trudeau Society, through its Committee on Therapy, is anxious to obtain information confirming or disproving this impression, and desires to ask the cooperation of physicians throughout the country in collecting pertinent data, as outlined below. Since tuberculosis is a common chronic disease, it seems likely that many physicians have had an opportunity to observe the effects of ACTH or cortisone on tuberculous infection coincident with the treatment of other disease states. The Committee believes that this information is of sufficient importance to make such a study worth while, for if suitable material can be collected it would provide information of considerable significance which otherwise might not be available for years.

It is understood, of course, that the Committee in no way wishes to interfere with the publication of results by individuals contributing to this study, and will respect any restrictions in the use of material.

Information is desired concerning both pulmonary and extrapulmonary tuberculosis as to whether any clinician has observed any of the following:

(1) no apparent effect in patients with known active tuberculosis; (2) an exacerbation of clinically active or apparently inactive tuberculosis during or after treatment with ACTH or cortisone; (3) a favorable effect in active tuberculosis.

If such cases are available, the following data would be greatly appreciated:

(a) a brief clinical history, including a statement concerning the disease for which treatment was given, and whether or not active or inactive tuberculosis was suspected before treatment was begun; (b) pertinent laboratory data, including results of sputum examination for tubercle bacilli; and (c) the temporary loan of representative X-rays related to the tuberculosis, if available, before, during, and after treatment. This information should be sent to Dr. Robert H. Ebert, University of Chicago Clinics, 950 E. 59th St., Chicago 37.

We shall appreciate having the opportunity to call this appeal to the attention of the readers of your Journal.

Sincerely yours,

WILLIAM B. TUCKER, *Chairman*

COMMITTEE ON THERAPY
AMERICAN TRUDEAU SOCIETY
UNIVERSITY OF MINNESOTA
MINNEAPOLIS 14, MINNESOTA

August 17, 1951

Books

PIONEER DOCTOR. First edition. By Lewis J. Moorman. Pp. 252, 12 illustrations, University of Oklahoma Press, Norman, Oklahoma, 1951, Price, $3.75.

In this book a well-known American physician, who has devoted the best years of his busy life to the understanding and control of tuberculosis, tells in a simple style the story of his seventy-five years. Seven of the twenty-seven chapters are devoted to tuberculosis. Five of them set forth in scholarly fashion the history of the disease and man's progress toward its conquest. Two are concerned largely with his own experience; they furnish a fascinating picture of the care of tuberculosis under pioneer conditions far from hospitals and long before the days of collapse therapy and streptomycin.

The rest of the book is autobiography and reflection. Born on a Kentucky farm, graduated from the Medical School of the University of Louisville a half-century ago, Doctor Moorman began practice in the little prairie town of Jet on the northwestern fringe of the Oklahoma Territory. Thirty years later he was Dean of the School of Medicine of the State University, and in the succeeding years held many positions of honor and trust in the medical profession, including the presidencies of the Southern Medical Association and the National Tuberculosis Association. The story of those years is told with all modesty in this volume. It is the record of a cultured physician, but lying between the lines is the unintended revelation of a kindly, scholarly, witty, but at all times humble, people's doctor who saw medicine's greatest progress with his own eyes and took an active and vigorous part in it.

ESMOND R. LONG

HELP YOURSELF GET WELL. By Marjorie McDonald Pyle. Pp. 235, Appleton-Century-Crofts, Inc., New York, 1951, Price, $3.00.

This book fills a need and fills it well. While much has been written for the benefit of the tuberculous patient, there have been several notable deficiencies in the past.

In the first place, recent changes in treatment methods and a vast improvement in outlook have necessitated a restatement of the problems of taking the cure. Other older books have frequently failed to lend themselves to effective use by the families of patients. Too often undue fear has been promoted by what has been written for patients. Previous books have all contained one or more statements considered false at the time they were read by this reviewer.

No disagreement could be found with the many facts presented in this important book. The only real criticism was a recurrent subtle implication that it frequently takes several years in a sanatorium to get well.

Stress has correctly been placed on the importance of education, psychology, mental as well as physical rest, and wide variations in treatment, all capable of achieving the same successful goal.

This reviewer takes exception to the opinion that *most* ex-patients must *always* preserve some caution. Many patients who have attained a good arrest of limited amounts of tuberculosis have apparently been able to forget their caution after three or four years.

The reaction of Trudeau Sanatorium patients to this volume was most informative.

All have apparently been able to read it carefully and without acquiring new fears. In fact, most have seemed comforted by the experience.

So many little situations common to tuberculous patients are well appreciated by the author: failure to really accept the verdict; rationalization that the diagnosis was a mistake; the difficulties with the attitudes of families; the importance of laying aside ambition for a time; the common mistake of putting a time limit on a cure; and old-timers waiting to torture the sanatorium neophyte.

A fair proportion of the book is devoted to an expert restatement of several matters fundamental to a book of this kind. The importance of occupational therapy and rehabilitation, planning the day, music therapy, hobbies, rules for ex-patients, a chapter on famous people who have had the disease, and the importance of "acquiescence" (to use Dr. E. L. Trudeau's own expression) are all well presented.

In conclusion, the book deals both factually and optimistically with the problems of tuberculosis treatment from the standpoint of the patient, his family, and the conscientious physician.

ROGER S. MITCHELL

BOOKS RECEIVED

TUBERCULOSIS AMONG CHILDREN AND ADULTS. Third edition. By J. Arthur Myers. Pp. 897, with illustrations, Charles C Thomas, Springfield, Illinois, 1951, Price, $12.50

BACTERIAL POLYSACCHARIDES. By Martin Burger. Pp. 273, Charles C Thomas, Springfield, Illinois, 1950, Price, $6.00

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American Trudeau Society

Medical Section of the National Tuberculosis Association

NOTES

ANNUAL MEETING—1952

The American Trudeau Society, medical section of the National Tuberculosis Association, invites the submission of scientific and clinical titles for presentation at its next annual meeting in Boston, May 27–30, 1952, at the Hotel Statler.

Abstracts of titles for this 1952 meeting, or completed papers, must reach the Chairman, Medical Sessions Committee, American Trudeau Society, 1790 Broadway, New York, not later than January 1, 1952.

Abstracts should be limited to 300 words at the most.

All abstracts and papers will be reviewed by the Committee for selection of the most interesting material. Papers not selected for reading on the program will be presented by title and read if time permits.

Subjects for presentation will include all clinical and scientific aspects of tuberculosis, nontuberculous respiratory and cardiac diseases as well as the results of investigative work with some bearing upon these subjects.

THE AMERICAN REVIEW OF TUBERCULOSIS

ABSTRACTS

Edited by LAWRENCE B. HOBSON

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CLINICAL STUDIES, TUBERCULOSIS

Glucose Content of Serous Pleural Effusions.—Bacteriologic confirmation of the tuberculous origin of nonpurulent pleural effusions may be difficult to obtain. Experience gained from glucose estimations upon the cerebrospinal fluid during tuberculous meningitis directed attention to the possible diagnostic value of the glucose content of pleural fluid. Among 61 cases of serous pleural effusion examined, 25 were considered tuberculous, the diagnosis being confirmed by bacteriologic examination of either sputum or pleural fluid or at necropsy; 6 cases remained undiagnosed. Of the 30 nontuberculous cases, 13 were due to congestive heart failure, 7 to carcinoma, one to leukemia, 2 followed pulmonary infarction, one was associated with ascites and pancreatitis, and 6 were postpneumonic. In many cases, the blood sugar was measured concomitantly and was normal; there was no correlation between the blood and pleural fluid glucose levels. Using the Hagedorn-Jensen method, each of the 30 nontuberculous pleural effusions contained more than 60 mg. of glucose per 100 ml., and 24 of these contained more than 100 mg. of glucose per 100 ml. In the 25 tuberculous cases, 13 effusions contained less than 60 mg. of glucose per 100 ml. A glucose level below the latter concentration strongly suggests a tuberculous etiology of the effusion and a glucose content of more than 100 mg. per 100 ml. probably indicates a nontuberculous etiology.—*Diagnostic value of the glucose content of serous pleural effusions, W. L. Calman, B. J. O. Winfield, M. F. Crowley, & A. Bloom, Brit. M. J., June 2, 1951, No. 4717: 123a.*—(*E. A. Riley*)

Tuberculoid Granuloma Associated with Carcinoma.—Sarcoid-like lesions have been found in lymph nodes receiving lymph from an area containing a malignant neoplasm. The well-known coexistence of neoplasia and granulomatous inflammation may be fortuitous but occasionally neoplasia develops as a complication of chronic granulomatous inflammation. More often, a granulomatous reaction results from irritant products, pressure or necrosis associated with growth of a neoplasm or with the action of therapeutic agents upon it. Such granulomas are often tuberculoid and, particularly in lymphoid tissues, may so resemble the lesions of sarcoidosis that the latter is erroneously diagnosed. Four instances of tuberculoid lesions without necrosis were found in lymph nodes receiving the lymphatic drainage from organs which were the primary site of carcinoma: the breast in 2 cases, the thyroid gland in one, and a bronchus in the other. In a fifth case, tuberculoid granulomas were present in the intrapulmonary lymphoid follicles in the vicinity of an invasive bronchial "adenoma." The granulomatous lesions are considered to have been the result of absorption of irritant materials from the tissues drained by the affected lymphoid tissue, and not a manifestation of a granulomatogenic infection or of the presence of any generalised disorder of the lymphoreticular system. Intracellular asteroid bodies were found in the granulomatous lesions in all cases, and paramyloid material was present in the lymph nodes in one case. Apart from the demonstration of tuberculin sensitivity in one case, no clinical or radiologic evidence of tuberculosis was found in any of the 5 cases. In one case, scanty acid-fast bacilli were found in many specimens of sputum but no tuberculous lesions were found in the excised lung.—*Localized tuberculoid granuloma associated with carcinoma: Their relation to sar-*

coidosis, W. St. C. Symmers, Am. J. Path., May-June, 1951, 27: 493.—(J. S. Woolley)

Tuberculous Lymphadenitis.—Among 51 consecutive cases of tuberculous lymphadenitis in Nigerian Negroes, 32 occurred in male patients. Only 17 of the patients were more than 20 years of age. In only 2 instances was there a history of tuberculous contact. Roentgenographic evidence of a pre-existing primary pulmonary complex was found in 35 per cent of the cases as compared with 21 per cent in a series of 100 comparable controls. In 30 of the 51 cases, more than one group of glands was involved; as a rule one group was primarily involved with subsequent spread to other groups. Although the cervical glands were most often initially affected, axillary nodes were primarily involved in 7 instances and inguinal nodes in 3. A tendency toward massive bilateral cervical glandular involvement was noted in children less than five years of age. Cold abscesses and sinus formation occurred in the younger age groups usually after prolonged disease but appeared in the older age groups often after disease of much shorter duration. Active concomitant pulmonary tuberculosis was present in 3 patients; 2 children had concomitant pleural effusions. The treatment was generally conservative and surgical excision was reserved for those cases in which sinuses developed or in which there was slow response to conservative measures. The probable pathogenesis is hematogenous dissemination from the primary complex to the peripheral nodes where the bacilli remain latent until some precipitating factor provokes overt tuberculous lymphadenitis.—*Tuberculous lymphadenitis in the Nigerian African, B. S. Jones, Brit. M. J., May 12, 1951, No. 4714: 1056.—(E. A. Riley)*

Cavity Behavior in Tuberculosis.—One may assume at least two important cases of cavity persistence of a mechanical nature: (*1*) cavity fixation, meaning rigidity of the cavity wall due to fibrosis and adherence of the surrounding lung to the resistant bony parietes; (*2*) cavity inflation, meaning the effects of a stop-valve in the draining bronchi. Based on these principles, a procedure was tried in 15 patients, in 13 of whom the cavity was 5 cm. or more in diameter, and practically all of whom had bilateral disease. Anterior thoracoplasty was first done with an extensive apicolysis. Re-expansion of the lung was then allowed for eight to twelve weeks or longer. In one group of 5 cases, the cavities completely disappeared. In a second group the cavities diminished and then remained constant. Further compression was provided by a posterior stage. Eight of 16 cavities studied were of this type, and 5 were obliterated. In the third group there was re-enlargement of the cavities after the initial diminution following the anterior thoracoplasty. These were treated by lung resection, or if resection were inadvisable because of extensive disease, by transpleural drainage. Two cavities were of this type and remained obliterated for three and four years after the outlined treatment. No valid statistical conclusions can be drawn from a group of 16 cavities in 15 patients, one patient having been operated upon bilaterally. It is hoped that others may adopt similar studies in cavity behavior.—*Some aspects of cavity behavior in pulmonary tuberculosis, G. A. Hurley, J. Thoracic Surg., August, 1950, 20: 325.—(R. E. MacQuigg)*

Pleuritic and Pulmonary Tuberculosis.—Among 136 patients with tuberculous pleurisy diagnosed during 1938 through 1941 and followed for periods ranging from eight to eleven years, there were 69 men and 67 women. Approximately two-thirds of each sex fell in the age group 15 to 29; none was more than 60. Only 21 (15 per cent) subsequently developed some form of tuberculosis; 17 had pulmonary tuberculosis; 10 of these had negative roentgenograms during the pleurisy. The blood sedimentation rate may have some prognostic value but the quantity of the exudate apparently does not. Because of the seriousness of the syndrome, prophylactic BCG vaccination is recommended.—*Pleuritis Tuberculosa en Longtuberculose, B. van Vliet,*

Nederl. tijdschr. v. geneesk., March 3, 1951, 95: 684.—(J. van der Vate).

Bronchial Changes After Primary Tuberculosis.—Marked residual lesions of the bronchi are often found following primary pulmonary tuberculosis. They are mostly asymptomatic and occur without any or with slight changes in the chest roentgenograms. Of 101 patients with lobar or segmental shadows, 51 were followed; 30 had bronchial lesions one to sixteen years after the discovery of the original shadow. The principal findings were stenoses, ectasia, severe shrinking, and marked growth. With one exception these lesions were found in the upper lobes, the middle lobe, and the lingula. They did not give rise to any symptoms except in one patient with both stenosis and ectasia of the lower lobe. The residual shadows in right middle and left upper lobe are probably often due to a large extent to pleural thickening.—*De Mogelijke Gevolgen van Primaire Longtuberculose voor De Bronchi, G. M. H. Veneeklaas, Nederl. tijdschr. v. geneesk., April 28, 1951, 95: 1295.—(J. van der Vate).*

Pulmonary Tuberculosis and Carcinoma.—In a series of 86 cases of pulmonary and bronchial carcinoma, observed between January 1948 and June 1949, 9 cases were associated with active pulmonary tuberculosis. In 5 of these there was no evidence of etiologic relationship between tuberculosis and neoplasm. In 4 other cases the histologic examination revealed that the neoplastic process arose out of old tuberculous scar tissue. The tumors were small and peripherally situated and were for these reasons difficult to diagnose. Death occurred in these cases as a consequence of early and widespread metastases.—*Gleichzeitiges Vorkommen von Lungentuberkulose und Lungenkrebs, E. Muntean & R. Amon, Fortschr. Röntgenstrahl., June, 1950, 73: 156.—(L. Molnar)*

Sterility in Genital Tuberculosis.—Curettage of the uterus of sterile Dutch women frequently shows tuberculous infection. Tuberculous endometritis is a rather common affliction, readily verified by guinea pig inoculation using blood of the first- or second-day menstrual discharge. This procedure is indicated in sterile women; those with vague abdominal complaints, even if nothing abnormal is detected by palpation of the internal genital organs; those with palpable anomalies of unknown etiology; those with suspected tuberculosis elsewhere in the body; and in women being treated for abdominal tuberculosis. Tuberculous endometritis probably is seldom accompanied by pulmonary tuberculosis; it is more frequent in the presence of or following extrapulmonary tuberculosis, including pleurisy, and especially after tuberculous peritonitis. Although the latter often appears to heal spontaneously, it should not be considered cured so long as the menstrual blood remains positive. Antibiotic treatment with para-aminosalicylic acid and streptomycin results in considerable improvement, but whether permanent recovery can be obtained is still uncertain.—*Diagnostiek en Therapie van Tuberculose van Organen Onder in De Buik, J. B. Stolte & T. Castelein, Nederl. tijdschr. v. geneesk., February 10, 1951, 95: 460.—(J. van der Vate).*

Diaphragm in Pregnancy.—A method of measuring the height of the diaphragm under standard conditions was devised. A control group of 30 non-pregnant women of childbearing age was compared to a group of 19 pregnant women. A slight but significant rise in the level of the diaphragm occurred in the later months of pregnancy. In neither group was there a significant difference in the range of variation between the left and right sides. It is not believed that the changes after delivery have much influence on pulmonary tuberculosis. The decision as to the treatment of tuberculosis in pregnancy is determined by the status of the tuberculosis. A pneumoperitoneum can be induced and maintained until the thirty-second week. A pneumothorax may be induced up to the seventh month. There is no reason for reinducing pneumoperitoneum immediately after delivery. Phrenic paralysis

results in atrophic changes in the diaphragm. For this reason, it should be avoided in pregnancy which, by stretching, can cause permanent changes in the organ.—*The diaphragm in pregnancy, C. J. Stewart, Tubercle, February, 1951, 31: 40.*—(*A. G. Cohen*)

CLINICAL STUDIES, NONTUBERCULOUS DISEASES

Coccidioidomycosis Cavities.—Primary pulmonary coccidioidomycosis tends to leave residua such as single or multiple solid foci, coccidioma, cavitation, hilar adenopathy, calcification, and localized bronchiectasis and fibrosis. Residual cavities vary in size from 1 to 14 cm.; they produce hemoptyses in 65 per cent of the patients. Cavities closed spontaneously in 25 per cent of 92 patients, they persisted in 10 per cent without harm, they filled and formed solitary foci in 6 per cent, and they ruptured through the pleura in 2 per cent. Collapse therapy by pneumothorax or pneumoperitoneum may close a cavity. Thick-walled cavities may be removed by simple excision; larger cavities, by segmental resection, lobectomy, or pneumonectomy. There has been no fatal dissemination of disease from a pulmonary cavity. Transmission of the infection from one person to another does not occur.—*Pulmonary mycoses—Coccidioidomycosis and pulmonary cavitation: A study of ninety-two cases, W. A. Winn, A. M. A. Arch. Int. Med., April, 1951, 87: 541.*—(*G. C. Leiner*)

Pulmonary Sarcoidosis.—The three stages of pulmonary changes described by Gravesen in sarcoidosis are: (*1*) hilar lymph node enlargement, (*2*) miliary dissemination, and (*3*) thickening of the pulmonary markings due to sclerosis of the lymph vessels. These were studied in 66 cases. In about one-sixth of the cases a further stage was observed, characterized by nodular infiltration, mostly in the lateral areas of the middle lung fields, and emphysema. In 2 cases a progressive clinical course was noted with insistent cough, severe dyspnea, loss of weight, and extensive fibrosis with gross nodules in both lung fields. Histologic examination of lymph nodes obtained by biopsy confirmed the diagnosis in 16 cases. In about one-third of the cases cystic bone changes, characteristic for sarcoidosis, were found. Uveoparotiditis was observed in 7 cases and splenomegaly in 21 cases. The intradermal reaction to 1:10 and to 1:100 Old Tuberculin was negative in all of the histologically proved cases.—*Über den Verlauf der Boeckschen Erkrankung der Lungen, H. Hartweg, Deutsche med. Wchnschr., August 11, 1950, No. 31/32: 1013.*—(*L. Molnar*)

Fibrocystic Disease of the Pancreas.—Following a study of 102 cases of fibrocystic disease of the pancreas in children, it was discovered that 2 groups of symptoms were evident. The first group, consisting of cough, recurrent pneumonia, and wheezing, arose from pulmonary complications. The second group of symptoms were related to the gastrointestinal tract. When first seen, 72 per cent of the patients showed a predominantly respiratory involvement. Approximately one-third of the histories were of respiratory disease without any suggestion of a nutritional disorder. The most significant physical findings were in the chest, 80 per cent of the patients showing abnormal physical signs at some time. The most common findings were rales, rhonchi, and signs of obstructive emphysema. Clubbing, which had been present in most children for more than a year, appeared with equal frequency in the respiratory and nutritional types of cases. Roentgenographic examination of the chest in 71 patients revealed emphysema in 93 per cent and increased peribronchial markings in 87 per cent. Atelectasis and patchy areas of pneumonia were also frequent. Several chest films were similar to those seen in miliary tuberculosis. The differential roentgenographic diagnosis included asthma, bronchiectasis, bronchial obstruction with atelectasis and emphysema, and miliary tuberculosis. Treatment of the respiratory complications consisted of penicillin inhalation three times daily or the use of other antibiotics when resistance to penicillin developed. It is of importance that this disease be considered when

problems of "chronic bronchitis" in infancy and early childhood arise since early treatment may favorably influence the outcome.—*Fibrocystic disease of the pancreas, V. Abbott, J. F. McCreary, R. Pocock, & H. Brown, Canad. M. J., May, 1951, 64: 419.—(E. A. Riley)*

Emphysema.—The purpose of the investigation was to test the claim that emphysema presents a characteristic radiologic appearance. Twenty patients with emphysema were selected for study as were 20 normal controls of comparable age. Postero-anterior and lateral chest roentgenograms were taken in both full inspiration and expiration. They were reviewed by four different expert observers with the following criteria in mind: increased radiolucency of lung fields, increased retrosternal space, bullae, wide rib spaces, increased vascular markings, increased hilar markings, enlargement of the pulmonary conus, kyphosis, horizontal ribs and calcification of the costal cartilages. Many of these individual features were noted in normal persons of late middle age or over. When based only on a postero-anterior film in full inspiration, the diagnosis of emphysema was very uncertain. A more reliable diagnosis was made when all four films were included in the study. Even so, approximately 10 per cent of cases were missed and a few normal persons were erroneously diagnosed as having emphysema.—*Radiological diagnosis of emphysema, J. M. S. Knott & R. V. Christie, Lancet, April 21, 1951, 1: 881.—(A. G. Cohen)*

Lipoid Pneumonia.—Lipoid pneumonia, first thought to occur only with chronic or disabling ailments, has been observed in apparently healthy individuals. Recent reports indicate that the introduction of oils into the nose and throat frequently produces chronic pulmonary lesions. Despite normal cough and swallowing reflexes, a surprisingly large number of patients aspirated oil into the lung. Mineral oil introduced into the pharynx can enter the bronchial tree without exciting reflex responses. In animals liquid petrolatum fails to produce a cough reflex and does not stimulate reflex closure of the glottis. In addition it hinders ciliary activity mechanically by slowing or stopping the flow of mucus. Progression of the disease after discontinuance of the incitant oil may be due to re-aspiration of disintegrated lipophages. The great variation in the clinical picture of this disease in adults makes the differential diagnosis difficult. The roentgenographic findings are frequently atypical and inconclusive. A survey of 389 chronically ill patients revealed 57 cases (14.6 per cent) of lipoid pneumonia diagnosed by (*1*) examination of the sputum, (*2*) aspiration from the lung, and (*3*) roentgenographic studies of the chest. In 55 patients the sputum showed characteristic lipophages with abundant free lipoid material; aspiration from the lung revealed typical lipoid-containing macrophages. Of the 20 patients with characteristic roentgenographic manifestations of lipoid pneumonia, 13 had retrocardiac changes revealed in lateral, oblique, and overexposed films. Occasionally the overexposed film was diagnostic of oil-aspiration pneumonia when the routine postero-anterior films were negative for such lesions. A significant number of the patients had a productive cough and positive physical signs which were most frequent over the base of the right lower lobe. Most of these patients had used mineral oil as a laxative. Others had used nose drops or various oil-containing medications.—*Incidence of lipoid pneumonia in a survey of 389 chronically ill patients, B. W. Volk, L. Nathanson, S. Losner, W. R. Slade, & M. Jacobi, Am. J. Med., March, 1951, 10: 316.—(T. H. Noehren)*

Bornholm Disease and Coxsackie Virus.—Among 6 patients with clinical histories and findings compatible with Bornholm disease or epidemic pleurodynia, Coxsackie virus was isolated from one and a rising agglutination titer against this agent was present in 4 of the 5 patients who were available for testing. Four of these cases occurred in the same family.—*Bornholm disease, T. Geffen, Brit. M. J., May 26, 1951, No. 4716: 1185.—(E. A. Riley)*

ACTH in Chronic Lung Disease.—Studies of the effect of adrenocorticotrophic hormone (ACTH) on the clinical course, pulmonary function, and some metabolic aspects were carried out in 5 patients suffering from chronic lung disease. Two of the 5 patients exhibited striking improvement while receiving ACTH. There was return to normal of nearly all the derangements in pulmonary function, with prompt regression to their previous state on discontinuance of ACTH. One of these patients was a middle-aged man with advanced pulmonary emphysema, fibrosis, seasonal asthma for eighteen years, and status asthmaticus for the past two years. The other was a young woman with Boeck's sarcoid. Unfortunately this patient's course was complicated by the development of mental depression which culminated in suicide after ACTH injections were stopped. Two other patients became worse during ACTH administration. This response was thought to be related to fluid retention which taxed their already diminished cardiac reserve. One was a middle-aged man with asthma complicated by pulmonary fibrosis, emphysema, and cor pulmonale since childhood. The other was an elderly man with advanced pulmonary emphysema. The fifth patient, an elderly man who suffered from pulmonary fibrosis, bronchiectasis, and emphysema secondary to old inactive tuberculosis, exhibited neither benefit nor deleterious effect from prolonged administration of the hormone. Consistent changes in the pattern of plasma electrolytes and glucose tolerance were not noted (Authors' summary).—*Effect of ACTH in chronic lung disease, J. Galdston, S. Weisenfeld, B. Benjamin, & M. B. Rosenbluth, Am. J. Med., February, 1951, 10: 168.*—(*T. H. Noehren*)

ACTH for Eosinophilic Pneumonia.—In a case of protracted pulmonary disease a diagnosis of eosinophilic pneumonia was made. Administration of ACTH resulted in dramatic improvement which has persisted for over a year.—*Treatment of eosinophilic pneumonopathy, R. W. Vines & D. Clark, New England J. Med., May 31, 1951, 244: 826.*—(*A. G. Cohen*)

Sinobronchitis in Children.—The medical records of 385 children who were admitted to a Pittsburgh hospital with a complaint of chronic respiratory infection were correlated with the roentgenograms of the nasal sinuses and the lungs. The prevalence of demonstrable sinobronchitis in this group was 37.7 per cent, in contrast to a 7.7 per cent incidence in a control group with no symptoms.—*Sinobronchitis in infants and children, W. C. Price, J. Pediat., May, 1951, 38: 590.*—(*W. H. Oatway, Jr.*)

Spontaneous Hemopneumothorax. — The occurrence of bleeding into the pleural space after spontaneous pneumothorax may result in an inexpansible lung but also may result in a 25 per cent mortality rate. A case is reported in which thoracotomy was used. A large volume of blood was removed, the bleeding was stopped, the lung was re-expanded at once, and recovery followed three days of constant closed negative pressure by thoracotomy tube.—*Spontaneous hemopneumothorax, R. R. Myers, F. R. Johnston, & H. H. Bradshaw, Ann. Surg., March, 1951, 133: 413.*—(*W. H. Oatway, Jr.*)

Venous Air Embolism.—Air enters veins under such different circumstances as pregnancy and its complications; diagnostic or therapeutic dilation and curettage; tests for tubal patency; retroperitoneal trauma; operations on pelvic, retroperitoneal, cervical, or cranial structures; the injection of air into the pelvic organs, or into the peritoneal, pleural, retroperitoneal, or subarachnoid space; and the administration of intravenous infusions or transfusions. Air enters an incompletely collapsed vein as a result either of negative venous pressure or of high extrinsic positive pressure. Air in the right ventricle and pulmonary arteries is compressible while blood is not, and the contractions of the ventricle are ineffectual in propelling the blood through the capillary bed. The best method of treatment of serious air embolism is to remove the intravascular air at once. This is best accomplished by introducing a needle below the xiphoid

process or in the fifth intercostal space just to the right of the sternum. The needle should be directed upwards after entering the heart to allow the floating air to enter it. A rapid diagnosis is of paramount importance. Embolism should be suspected in any patient developing sudden cardiac arrest on the operating table and the heart area should be auscultated for the "mill-wheel" murmur. Other suggested methods of treating air embolism include inhalation of pure oxygen and placing the body on its left side so that the air bubbles float above the blood level. In 4 fatal cases of air embolism observed by the author, prompt aspiration of the air from the right ventricle might have been life saving.—*Venous air embolism, C. P. Larson, Am. J. Clin. Path., March, 1951, 21: 247.*—(*J. S. Woolley*)

Pulmonary Embolism.—In a five-year period, 24 cases of pulmonary embolism were seen; approximately one-half were verified at necropsy. There were 21 cases (0.44 per cent) of such embolism in a series of 4,766 major operative cases. There were 16 female and 8 male patients but there were twice as many female patients as male patients operated upon. The age of the patients was four to eighty years. In the postoperative cases, embolism occurred 7.8 days postoperatively on the average, with a range from the first to the twelfth day.—*Pulmonary embolism: A review of 24 cases in a five-year period at St. Mary's Infirmary, E. A. Lee, J. Nat. Med. Assoc., May, 1951, 43: 181.*—(*B. Hyde*)

Treatment of Empyema.—In 12 consecutive cases, when pus or infected fluid was found in the pleural cavity, as complete aspiration as possible was done and 500,000 units of penicillin were introduced. Subsequent treatments were given at one- to three-day intervals until no pus could be obtained. In addition, 200,000 units of penicillin were given parenterally at eight-hour intervals. Rapid improvement was noted after the first treatment. The temperature usually became normal in approximately fourteen days. The pus became sterile in approximately seven days, and disappeared after an average of forty days. On follow-up, the lungs on the affected side were found to have expanded completely.—*Empyema thoracis treated with penicillin and aspiration only, R. Asher & D. Davies, Lancet, April 28, 1951, 1: 924.*—(*A. G. Cohen*)

Tracheo-Esophageal Fistula.—Vogt and Ladd described 5 types of tracheo-esophageal fistulas. Most of the reported cases have had a closed proximal esophageal pouch with a fistula between the distal esophageal segment and the tracheobronchial tree. The authors report a rare and unclassified anomaly; the unstenosed esophagus was connected with the trachea by a fistula. It was associated with coughing after food ingestion, recurrent pneumonia, and meteorism. Surgery was effective in correcting the defect.—*Congenital tracheo-esophageal fistula without esophageal artesia, J. A. Helmsworth & C. V. Pryles, J. Pediat., May, 1951, 38: 610.*—(*W. H. Oatway, Jr.*)

Surgery for Pulmonary Carcinoma.—From 1930 to 1950 a presumptive diagnosis of carcinoma of the lung was confirmed by histologic evidence in 681 patients. Of these proved cases, 294 were subjected to thoracotomy and resection was done in 171, 58.2 per cent of those explored. Of these, 137 left the hospital alive. At the time of this report, of the 87 patients who underwent total pneumonectomy before 1948, 19 (22 per cent) survived. Of the 31 patients who underwent lobectomy in the same period, 8 (26 per cent) were alive. In the past two years the operative mortality for both pneumonectomy and lobectomy has been less than 4 per cent. In discussing this paper Aufses stated that, of 34 lobectomies for carcinoma at Mt. Sinai Hospital, 38 per cent were well; and of 52 pneumonectomies, 17 were well. Hudson cited an 18 per cent survival rate in 83 pneumonectomies; whereas of 31 lobectomies, 25 per cent survived. DeBakey pointed out that the fact that a lobectomy was done excluded more advanced cases. He stated that the resectability rate among his private cases was 3 or 4 times greater than

among the charity cases. Allison pointed out that pneumonectomy was not well defined in the discussion and that many simple excisions of the lung were in no wise superior to lobectomy, removing much good lung tissue without a more radical attack on the malignancy. To do either a lobectomy or pneumonectomy and then pick out isolated glands from the mediastinum is to forget surgical pathology. All tissues removed should be removed in continuity. He divides the pulmonary vessels within the pericardium and removes the pericardium adjacent to the lung root with its mediastinal cellular tissue and glands attached.—*The surgical management of carcinoma of the lung, E. D. Churchill, R. H. Sweet, L. Soutter & J. G. Scannell, J. Thoracic Surg., September, 1950, 20: 349.*—*(R. E. MacQuigg)*

Bronchogenic Carcinoma.—Among 94 cases of bronchogenic carcinoma diagnosed by autopsy, biopsy, or cytologic examination, 82 occurred in men whose age averaged 62 years. The right main bronchus was the most common site of origin followed by the left and then the right upper lobe bronchi, the upper lobe bronchi accounting for 36 per cent of the total. In 71 cases in which it was possible to establish an approximate time interval, the average time between the first symptoms and a positive diagnosis was seven months. In only 30 per cent of the cases was the diagnosis within three months of the first symptoms and by the end of six months only 60 per cent were diagnosed. Among these 94 patients, thoracotomy was thought indicated in only 23; 7 of these patients were found to have inoperable lesions, 14 patients underwent pneumonectomy, and 2 lobectomy. Six postoperative deaths occurred. Only 4 of the resected cases were well at the time of writing, and only 2 of these had been followed for more than six months. The cure rate can be improved only by earlier presentation of the patient for pneumonectomy.—*Bronchogenic carcinoma: Analysis of ninety-four cases, J. D. Stenstrom, Canad. M. J., May, 1951, 64: 409.*—*(E. A. Riley)*

Multiple Primary Cancers.—Up to 1932, 1,259 instances had been reported of more than one primary cancer in the same individual. Warren and Gates found 194 instances of multiple primary malignancies in 2,829 autopsies on cancer patients, an incidence of 6.8 per cent. They concluded that an individual with one cancer is eleven times more apt to develop a second cancer than a patient without cancer is apt to develop his first lesion. They stated that an average interval of 3.2 years elapsed between the appearance of the individual neoplasms when this could be measured. Therefore in follow-up examinations, patients who have had cancer should be scrutinized for evidence of a separate primary growth as well as for metastases. In 1,493 cases of cancer of the lung observed in Memorial Hospital, New York, in a period of twenty-four years, there have been 25 instances in which the lung tumor was one of separate primary malignancies, an incidence of 1.6 per cent. There was one triple primary. Criteria for multiple cancers must be clearly defined. Evidence is positive when the tumors have entirely different histologic characteristics; 7 cases in this series meet this criterion. Another 13 cases are classed as having probable evidence of multiple primary cancers. In a separate group are 5 cases of lung cancer associated with basal cell carcinomas of the skin, which are nonmetastasizing. Diagnosis is difficult. Lesions discovered before symptoms develop are more likely to be resectable; a delay of even three weeks may convert a resectable to a nonresectable lung cancer. Of the 25 lung cancers in this series, 11 were surgically explored; 7 had pneumonectomies; 2, lobectomies; and 2 were considered nonresectable. Except for palliative benefits, roentgen ray therapy was of little value in the prolongation of life.—*Multiple cancers: Primary in the lung and other sites, W. G. Cahan, F. S. Butler, W. L. Watson, & J. L. Pool, J. Thoracic Surg., September, 1950, 20: 335.*—*(R. E. MacQuigg)*

Aspiration During Anesthesia.—A study of 300 unselected surgical patients was made

to determine the frequency of obvious or silent aspiration of the stomach contents during anesthesia. Evans blue dye was placed in the stomach by gastric tube or a capsule fifteen or thirty minutes before induction. The mouth, pharynx, larynx, and bronchi were inspected after operation. Regurgitation occurred in 26 per cent of the patients, and 16 per cent aspirated gastric contents into the lung. Frank vomiting occurred in only 8 per cent. Aspiration was twice as frequent from the gross emesis as from the silent. Regurgitation occurred more often in the Trendelenburg and lateral positions. It is more frequent in the patients handled by inexperienced anesthetists. Gastric tubes exert a protective effect by reducing the volume of gastric contents.—*Frequency of aspiration of gastric contents by the lungs during anaesthesia and surgery, G. A. Culver, H. P. Makel, & H. K. Beecher, Ann. Surg., March, 1951, 133: 289.—(W. H. Oatway, Jr.)*

Diaphragmatic Flutter.—A case of diaphragmatic flutter was at first diagnosed as paroxysmal tachycardia. Later, observance of the patient during attacks, supplemented by fluoroscopy, electrocardiography, and phonocardiography yielded the correct diagnosis. The condition may be asymptomatic. The most common complaint is breathlessness. Less common is pain at the site of the diaphragmatic attachment, the pain often simulating that of myocardial infarction. Examination during an attack shows epigastric pulsation at a rate of 70 to 200 per minute. Bruits may be heard which can be mistaken for cardiac sounds. Observation of the fluttering motion of the diaphragm fluoroscopically is characteristic. Electrocardiograms often show small rapid oscillations. The condition is seen in a variety of chronic diseases, particularly encephalitis lethargica. In many cases, it is due to a nervous tic. In treatment, phrenic crush has been used with varied degrees of success.—*Diaphragmatic flutter, C. G. Baker & A. B. Shaw, Lancet, May 6, 1951, 1: 985.—(A. G. Cohen)*

Anomalous Pulmonary Veins.—The literature now embodies 133 case reports of anomalous drainage of pulmonary veins. Of these patients, partial drainage into the right atrium occurred in 75 and complete drainage into this chamber or its tributaries occurred in 56. Additional cardiovascular anomalies were present in 23 of the latter group of patients. Partial drainage of pulmonary veins into the right atrium is consistent with long life. In 56 reported cases of complete drainage of pulmonary veins into the right atrium, only 10 patients lived beyond the age of eight months. The clinical diagnosis of complete drainage of pulmonary veins into the right atrium can be established by use of the cardiac catheter and the angiocardiogram. An additional case is described in which all of the pulmonary veins emptied into the superior vena cava in a male infant who lived to the age of eight weeks (Author's summary).—*Anomalous pulmonary veins, J. C. Smith, Am. Heart J., April, 1951, 41: 561.—(G.C. Leiner)*

Bronchography with Ioduron B.—In 1948, the Swiss discovered a water-soluble preparation, ioduron B, which was absorbed from the lungs following bronchography so that it was no longer visible on chest roentgenograms taken three to four hours later. Since it was alleged that there were no harmful residuals, it was thus possible to perform successive unilateral or bilateral bronchograms on the same patient. The opportunity presented itself to examine 6 lungs histologically which were resected six, fifteen, seventeen, twenty-three, forty-two, and ninety days after ioduron B bronchography. In each case residuals of the injected contrast medium were found to be present. Experimentation with rats showed that, although the ioduron is quickly absorbed, the vehicle carboxymethylcellulose remains in the lungs and can give rise to tissue reaction. The use of ioduron B, therefore, is not as harmless as originally proposed.—*Veranderungen des Lungengewebes nach Bronchographien mit Ioduron B, W. Vischer, Schweiz. med. Wchnschr., January 20, 1951, No. 3: 54.—(E. Dunner)*

Abnormal Substances in Sputa.—In many intrathoracic diseases an abnormal substance or body will occasionally be expectorated. The detection of this substance is not only of diagnostic significance but is often pathognomonic of that disease. Such substances include: caseous particles, broncholiths, carbon particles, asbestos bodies, foreign bodies, food, hair, bone, chyle, oil, fat, bronchial casts, Curschmann's spirals, Charcot-Leyden crystals, and malignant tumor masses. The following parasites may also be found: *Amoeba, Echinococcus, Ascaris, Strongyloides, Paragonimus westermanni, Syngamus trachealis,* and mites.—*Expectoration of abnormal substances and particles including parasites, A. G. Cohen, Am. J. Med., February, 1951, 10: 182.*—(*T. H. Noehren*)

ANTIMICROBIAL THERAPY

Delay in Diagnosing Tuberculous Meningitis.—The interval between the appearance of the initial symptom of tuberculous meningitis and the start of streptomycin therapy was known in 92 cases treated at the Sheffield Children's Hospital. Among the 77 children admitted with the diagnosis, the average interval was 14.2 days. An average of 3.3 days had elapsed before a physician was called, although in two-thirds of the cases the physician was called within 3 days. The greatest delay, averaging 8.3 days, occurred between the physician's first visit and admission to a hospital. Another 2.4 days on the average passed before the diagnosis was made in the hospital and the patient transferred for specific therapy. A further 1.2 days were consumed in substantiating the diagnosis at the Sheffield Hospital. The necessity for rapid treatment is shown by a mortality of 88.2 per cent when therapy was begun on the fifteenth day of symptoms or later, as compared to 47.8 per cent when treatment was started earlier. Only 2 (14.2 per cent) of 14 children died when meningitis appeared during the course of known tuberculosis and was treated without measurable delay. The survivors have been followed an average of 16.3 months from the start of treatment. To avoid unnecessary delay and so to improve the chances of successful treatment, physicians should be alerted to the possibility of tuberculous meningitis and taught to perform a tuberculin test when suspicious symptoms appear. A positive reaction warrants immediate hospitalization, prompt and rapid diagnostic procedures, and treatment without delay.—*The delay in the diagnosis of tuberculous meningitis and its relation to the results of treatment, J. Lorber, Proc. Roy. Soc. Med., January, 1951, 44: 1.*—(*L. B. Hobson*)

Streptomycin for Tuberculous Pleurisy.—Forty-seven patients with primary serofibrinous pleurisy were treated by removal of the fluid and instillation of streptomycin, 1 gm., in 20 cc. of physiological saline solution into the pleural space. In 27 cases no fluid reappeared after one treatment, in 16 cases after two treatments. In only 8 patients subfebrile temperatures persisted after the treatment. This method of therapy was unsuccessful in 4 cases. The age of the patient and the duration of the disease prior to the treatment had no relation to the effect of the treatment.—*Über die Behandlung der primären serofibrinösen Pleuritis durch totale Entleerung des Exzudates und Einspritzung von Streptomycin in den Pleuraraum, E. Danopoulos & K. Melissinos, Wien. klin. Wchnschr., May 4, 1951, 63: 333.*—(*G. C. Leiner*)

Streptomycin and Complications After Resection.—The incidence of complications following pulmonary resection for tuberculosis in a group of 60 cases reported in 1948 was compared with that in a new series of 146 patients, 22 of whom developed bronchopleural fistulas. There has been an increase in the number of immediate and late complications without any marked change in the mortality rate. In the earlier series, the operative mortality was 5 per cent; 7 per cent developed bronchopleural fistulas and empyema. In the new series, there were 8 fistulas following lobectomy and 14 after pneumonectomy, a percentage of 15; 14.5 per cent developed empyema. Comparison of cases with complica-

tions and those without shows: (*1*) The incidence of complications was unrelated to the indication for resection unless this was a destroyed lung. Of the patients developing fistulas, 22.7 per cent had a destroyed lung, whereas only 8 per cent of the total series had this condition. (*2*) Endobronchial disease did not appear to be a contributing factor to the development of fistulas. (*3*) Those patients with progressive tuberculosis and recent or exudative disease showed a higher proportion of complications (63 per cent) than that for the whole series (41 per cent). (*4*) Operative details, such as the suture material or method of stump closure, did not influence the incidence of complications. Pleural contamination was more serious, occurring in 54 per cent of the fistula group as opposed to 29 per cent of the total series. (*5*) The administration of an earlier course of streptomycin increased the number of postoperative complications, especially in those patients whose organisms were resistant to streptomycin at the time of resection. Complications were twice as frequent in the total series as in the original group of 60 cases. Spread or exacerbation was nine times as common in the total series. In those patients with previous streptomycin treatment, empyema was 4.5 times and fistula 6 times more frequent than in the previously untreated group. Spread and late deaths were also much higher in the treated group. Negative sputum was obtained in only 75.8 per cent of the previously treated, as compared to 87.8 per cent of the untreated patients. It has been suggested that increased prevalence of complications is because streptomycin has allowed operations to be performed on cases which previously had been rejected, but an analysis of the cases does not sustain this viewpoint.—*Complications following pulmonary resection for tuberculosis in streptomycin-treated patients, J. D. Murphy, H. E. Walkup, H. H. Seiler, & S. Bornstein, Dis. of Chest, May, 1951, 19: 493.*—(*E. A. Rouff*)

Streptomycin and Tuberculin Reaction.—From the results obtained in 912 patients undergoing streptomycin therapy it is concluded that: (*1*) The level of skin sensitivity to tuberculin was depressed in 65.8 per cent of the patients. These patients showed a clinically favorable response. The intracutaneous tests were performed after 15, 30, 50, and 70 gm. of streptomycin were administered. The depression of skin sensitivity occurred most frequently during the administration of the first 30 to 50 gm., which coincides with the most efficacious period of streptomycin therapy. (*2*) In 16 patients whose clinical response was not good, the tuberculin allergy remained stationary. (*3*) There was no appreciable change in 6 untreated and in 10 patients receiving *p*-aminosalicylic acid. Streptomycin-treated patients have a more sudden reversal in their disease process. (*4*) The depression of tuberculin sensitivity is not related to the streptomycin blood level but rather to the bacteriostatic effect on the tuberculous process. (*5*) There was no appreciable difference between the effect of streptomycin as compared with dihydrostreptomycin. It is concluded that a depression in the quantitative tuberculin reaction during the course of streptomycin therapy can be correlated with clinical improvement.—*Das Verhalten der Tuberkulinempfindlichkeit bei der Streptomycinbehandlung der Tuberkulose, J. Leitner, O. Wetterwald, & W. Beutl, Schweiz. med. Wchnschr., January 27, 1951, No. 4: 87.*—(*E. Dunner*).

PAS for Tuberculous Cervical Adenitis.—Over a period of ten years, 372 cases of tuberculous cervical adenitis have been treated by the following methods: curettage, 181; aspiration and ultra-violet irradiation, 75; aspiration and installation of "Promanide", 65; and aspiration and instillation of sodium *p*-aminosalicylic acid (SPAS), 51. The latter group is the subject of this article. Normally, the disease heals itself. The two shortcomings of this natural treatment are the disfiguring scars on the skin and the great length of time required for healing. Surgical treatment eliminates these drawbacks but hospitalization is required. The current treatment can be given on an outpatient basis. The abscess is aspirated and as much as 1 cc. of a 20 per cent solution

of SPAS is instilled. This procedure is repeated every week until the contents of the abscess become serous. If a sinus forms along the needle tract, a jelly containing 20 per cent of SPAS is instilled. If an ulcer forms, a cream containing the drug is applied. In 4 of 5 cases, tubercle bacilli were recovered. The ratio of human to bovine organisms was 5 to 3. There was very little tendency of the skin to break down and the pus become serous after three or four treatments. If a sinus or ulcer developed, it was soon eliminated. The healing time was less than with any of the previous methods.—*Treatment of tuberculous cervical adenitis with para-aminosalicylic acid, G. J. Norris & R. G. Prosser-Evans, Lancet, June 2, 1951, 1: 1199.*—(*A. G. Cohen*)

Anal Tuberculosis.—A patient with a tuberculous anal ulcer also had chronic fibroid phthisis. Treatment consisted of local application of streptomycin powder and oral administration of PAS. The ulcer healed within six weeks.—*Streptomycin in anal tuberculosis, R. Marcus, Lancet, May 12, 1951, 1: 1054.*—(*A. G. Cohen*)

PAS and Carinamide.—Elevation of the *p*-aminosalicylic acid (PAS) blood level can be obtained by increasing the dose of PAS or by blocking the renal tubules. Preparations to increase penicillin blood levels by blocking its excretion from the kidneys have included Diodrast, benzoic acid, carinamide, Benemid, and Basamid. High PAS blood levels were achieved by using 9 to 15 gm. of carinamide daily. Carinamide, orally, caused nausea and pyrosis; intramuscularly, it was painful; intravenously, it was tolerated satisfactorily. Blood PAS concentrations of 30 mg. per 100 ml. may be accompanied by emesis, vertigo, slurring of the speech, and slowing of the thought processes. These symptoms have also been observed as a result of a too rapid increase of the PAS blood level due to renal insufficiency. It has been reported that Benemid, 2 gm. daily in four doses, increases the PAS blood level fourfold. This method of increasing the PAS blood level may prove useful in serious forms of tuberculosis such as pneumonia, miliary, or meningitis.—*Tuberkulosebehandlung mit PAS unter Verwendung nierenblockierender Pharmaca, G. W. Lauenstein & W. Rockstroh, Tuberkulosearzt, February, 1951, 5: 74.*—(*E. Dunner*)

Intracavitary Drug Absorption.—The blood concentration and the elimination through the kidneys was studied after the instillation of sulfonamide derivatives into pulmonary cavities. The blood level showed a more rapid absorption when the cavity was thin-walled or when it was surrounded by richly vascularized recent infiltration. The rapidity of the absorption was similar to that obtained after intravenous or intraperitoneal administration. In general, 0.15 to 1 gm. of sulfonamide were instilled in 1.5 to 10 cc. of distilled water, or in a 10 per cent gelatin emulsion. The drug was still present in the blood ten hours after instillation of the watery solution, and seventy-two hours after the introduction of the gelatin emulsion. Amindan, a sulfonamide easily soluble in water, and irgafen powder were more rapidly absorbed than cibazol which is not easily soluble in water.—*Resorptionsverhältnisse in Lungenkavernen, W. Wilde & H. Fischer, Deutsche med. Wchnschr., July 28, 1950, No. 29/30: 976.*—(*L. Molnar*)

Treatment of Sinobronchitis.—The prevalence of sinobronchitis in children with chronic respiratory symptoms has been reported previously. New treatment programs were used for 54 consecutive patients admitted with this illness: 16 were treated with an antihistaminic drug and oral penicillin; 18 others were treated in the same way, as well as with aerosol penicillin; and 20 were additionally treated with decongestant-penicillin nose drops. The results in the third group were the best since shrinkage of the nasal membranes was required to allow the aerosol to enter the nose.—*A treatment program for chronic childhood sinobronchitis, W. C. Price & V. B. Binns, J. Pediat., May, 1951, 38: 597.*—(*W. H. Oatway, Jr.*)

LABORATORY STUDIES

Acid-fast Bacilli in Paraffin.—The presence of acid-fast bacilli in tissue sections usually establishes the diagnosis of tuberculosis provided the organisms are not introduced as a contamination. The tissue mat cubes used for infiltrating fixed tissues in preparation for sectioning have been found to contain acid-fast bacilli with morphologic and staining characteristics resembling those of *M. tuberculosis*. Prior to the discovery of this fact several erroneous diagnoses of tuberculosis were made. The organisms could not be grown in culture as the mat must be heated to 56° C. to melt it and the organisms, if they were viable, were apparently killed by this temperature. The morphologic and staining characteristics of the organism suggest that it may be *M. phlei.*—*Acid-fast non-tubercle bacilli in paraffin, O. J. Wollenman, Jr., P. O'B. Montgomery, & T. B. Foard, Am. J. Clin. Path., March, 1951, 21: 295.*—(*J. S. Woolley*)

Streptomycin-Dependent Mutants of Mycobacterium.—Two distinct colony types of streptomycin-dependent variants were isolated from a pigmented *Mycobacterium* recovered originally as a single colony from the gastric washings of a patient. These variants do not breed true but segregate into 6 different streptomycin-dependent mutants that do breed true to the new type. These latter are characterized by colony morphology, pigment production, and differences in growth rate. The reverse mutants derived from these streptomycin-dependent types in the absence of drug fall into 4 morphological groups: (*1*) large, golden, rough; (*2*) large, white, and rough; (*3*) small, smooth, and golden; and (*4*) small, smooth, and white.—*Heterogeneous character of streptomycin-dependent mutants of a Mycobacterium, D. Yegian & V. Budd, J. Bact., February, 1951, 61: 161.*—(*F. G. Petrik*)

Mutations of Streptomycin-Dependent Sulfathiazole-Resistant *M. Ranae*.—Nondependent variants were obtained from a streptomycin-dependent strain of *Mycobacterium ranae* isolated directly from the parent streptomycin-sensitive strain. Other nondependent variants were derived from a streptomycin-dependent strain selected from a sulfathiazole-resistant variant of this microorganism. The back mutations derived from streptomycin-dependent *Mycobacterium ranae* offer a new and promising approach in the study of bacterial genetics.—*Mutation of streptomycin-dependent Mycobacterium ranae selected from a sulfathiazole-resistant variant, D. Yegian & V. Budd, J. Bact., February, 1951, 61: 167.*—(*F. G. Petrik*)

Coproporphyrin III from Tubercle Bacilli.—Fluorescence and absorption spectral data obtained from an alcoholic extract of human tubercle bacilli indicated that coproporphyrin III is synthesized by the human tubercle bacillus. The emission and absorption band maxima are within the ranges reported for coproporphyrin III. Also the crystal forms and melting points conform to those of coproporphyrin III.—*Coproporphyrin III isolated from the human tubercle bacillus by chromatographic and fluorescence analysis: Fluorescence and absorption spectral data, M. O'L. Crowe & A. Walker, Brit. J. Exper. Path., February, 1951, 32: 1.*—(*H. J. Henderson*)

Biopsy Diagnosis of Tuberculosis.—The diagnosis of tuberculosis is frequently based upon histologic evidence rather than on the demonstration of the causative organism because it is claimed that *M. tuberculosis* produces a specific reaction in human tissues. Frequently, biopsy specimens are fixed immediately, rendering them useless for bacteriologic examination. To avoid this difficulty, frozen sections can be made so that granulomatous lesions can be examined bacteriologically or the surgeon must save and refrigerate a portion of the tissue in a sterile bottle for the bacteriologist. Many agents may give rise to histopathologic reactions like those of tuberculosis. For proper treatment it is desirable to isolate or identify the causative agent and, in some cases, to determine its sensitivity to chemical and antibiotic agents. Some of the shortcomings of histologic diag-

nosis of tuberculosis and some of the deficiencies of bacteriologic technique are shown by the following examples: (*1*) A lymph node removed from a man with multiple node enlargement was thought to demonstrate Hodgkin's disease. Numerous cultures were made for various organisms and *M. tuberculosis* was identified on Petragnani's and Löwenstein's media. (*2*) Lymph nodes from an 83-year-old woman histologically resembled Boeck's sarcoid. One of 2 guinea pigs inoculated with ground node material developed tuberculosis. (*3*) In another case, guinea pigs inoculated with granulation tissue from the wall of a purulent abscess developed tuberculosis. (*4*) Tuberculosis was suspected histologically in 3 cases extensively studied bacteriologically. One case was a *Brucella suis* infection, another was caused by *Coccidioides immitis*, and the third case was a beryllium granuloma with no pathogenic organisms present. The three major problems confronting the bacteriologist are: (*1*) the proper selection and method of collecting material suitable for bacteriologic study, (*2*) the collection of material before the patient has had extensive chemotherapy, and (*3*) the bacteriologic examination itself. If the patient presents a diagnostic problem and tissue is removed, it is suggested that the specimen be examined for the common organisms easily grown on blood agar, in brain broth, or in thioglycollate medium for tubercle bacilli, for *Brucella*, and for fungi. Guinea pigs are preferred to cultures for finding tubercle bacilli and occasionally reveal *Brucella* or *Coccidioides.—Problems concerned with the histological diagnosis of tuberculosis of lymph nodes, J. R. McDonald & L. A. Weed, Am. J. Clin. Path., March, 1951, 21: 223.—(J. S. Woolley)*

Carbon Distribution in Experimental Tuberculosis.—Small particles of carbon were introduced into the blood stream of animals developing experimental tuberculous lesions. Their distribution was studied particularly in tubercles at different stages of development. Ten rabbits were each inoculated intravenously with 0.05 mg. of a virulent bovine tubercle bacilli. Daily injections of carbon were begun four days after inoculation in the first rabbit and subsequently at two-day intervals in the other animals. Two control rabbits received carbon only and bacilli only, respectively. The rabbits died or were killed between the eleventh and sixty-third days after infection. All the inoculated rabbits except the one dying on the eleventh day had gross lesions in the lungs and spleen and usually in the liver and kidneys. Carbon particles were widely distributed through the tubercles if it was given within ten days after infection. Carbon was most plentiful in tubercles situated in tissues such as the hepatic parenchyma and the splenic pulp which store carbon and about vessels containing carbon thrombi. Tubercles or parts of tubercles growing in tissue where carbon is not stored tended to be carbon free. If carbon was given very early in the infection most of the cells which go to form the early lesions contained carbon before they took up tubercle bacilli. In such tubercles the carbon in the lesion was plentiful and diffuse; both carbon and tubercle bacilli were frequently observed in the same cell. When carbon was given some time after infection, the periphery of the spreading tuberculous tissue engulfed carbon-filled tissue macrophages. The epithelioid cells did not selectively take up carbon and generally contained less than the cells in the surrounding areas. The carbon was finely dispersed within such cells, in contrast with the heavy aggregates seen in macrophages. In rapidly spreading tuberculous tissue and in pneumonic areas in the lung, carbon was absent from the epithelioid cells. A few carbon-containing monocytes or macrophages seemed able to leave the vessels or their neighborhood and infiltrate into tuberculous tissue without undergoing any apparent change of form.—*The effect on experimental tuberculosis of the intravenous injection of insoluble substances: Experiments with carbon, N. P. Markham & H. W. Florey, Brit. J. Exper. Path., February, 1951, 32: 25.—(H. J. Henderson)*

Respiratory and Circulatory Reflexes.—Increased pressure in the perfused right side of the heart of the dog caused reflex bradycardia and peripheral vasodilatation from receptors in the right auricle. Increased pressure in the perfused pulmonary circulation caused a reflex peripheral dilatation and rapid, shallow breathing. Bradycardia resulted from increased pressure in the pulmonary arterial bifurcation. All of the presso-receptors are supplied by the afferent vagus. Although veratridine could still reflexly inhibit respiration and reflexly depress circulation when injected into the perfused lung and perfused heart, respectively, no chemo-reflex sensitive to changes in blood carbon dioxide and oxygen could be demonstrated in dogs. The pulmonary receptors sensitive to veratridine (Bezold reflex), like the pulmonary presso-receptors, appear to be in the pulmonary veins (Author's summary).—*Respiratory and circulatory reflexes from the perfused heart and pulmonary circulation of the dog, D. M. Aviado, Jr., T. H. Li, W. Kalow, C. F. Schmidt, G. L. Turnbull, G. W. Peskin, M. E. Hess, & A. J. Weiss, Am. J. Physiol., May 1, 1951, 165: 261.*—(*G. C. Leiner*)

Bronchial Arteries.—The lungs of 125 adult human subjects dead from various causes were injected, dissected, and radiographed. Four types of origin of the bronchial arteries predominated: (*1*) The commonest type, found in 40 per cent of the 125 subjects, was characterised by the presence on the right side of a single bronchial artery originating from a broncho-intercostal trunk behind the esophagus and by the presence on the left side of 2 bronchial arteries arising from the ventral aspect of the aorta. (*2*) The next most common type, present in 22.4 per cent of the 125 specimens, had a single bronchial artery on the right side arising directly from the ventral surface of the aorta in front of the esophagus and, on the left side, 2 arteries arising close together from the ventral aspect of the aorta. (*3*) In 17.5 per cent of the specimens there were, on the right, a single bronchial artery coming from the bronchointercostal trunk in front of the esophagus and, on the left side, 2 arteries arising close together from the ventral surface of the aorta. (*4*) In 12.8 per cent, there were two arteries, one, the superior bronchial artery arising from the bronchointercostal trunk back of the aorta; and, the other, the inferior bronchial artery coming directly from the ventral aspect of the aorta. If each lobar pedicle there were always anterior and posterior branches for each lobe. The bronchial arteries played an active part in the vascularization of every part of the visceral pleura. The cortical branches anastomosed with those arising deep in the lung tissue, producing a peripulmonary circulation originating in the bronchial arteries. There are also precapillary anastomoses between the bronchial arteries and the pulmonary veins, which may be indispensable and maintain life when the lung is diseased.—*Données nouvelles sur la circulation dans les artères bronchiques, M. Latarjet & P. Juttin, Poumon, January, 1951, 7: 35.*—(*A. T. Laird*)

PUBLIC HEALTH AND EPIDEMIOLOGY

Tuberculosis among Lima Children.—As determined by tuberculin testing, the tuberculosis infection rate among school children in the city of Lima, Peru, in 1943 to 1948 was 63.6 per cent: 62.4 per cent among boys and 65.2 per cent among girls. The figures for the Rimac suburb, among children examined in 1946 to 1948, were 52 per cent: 56.1 per cent for boys and 45.6 per cent for girls. Roentgen examination of the same groups showed tuberculosis lesions as follows: at Lima, 2.6 per cent: 2.9 per cent for boys and 2.1 per cent for girls; at Rimac, 2.3 per cent: 1.8 per cent for boys and 3.3 per cent for girls. There was a difference both in the total figures and relative involvement of the sexes in both groups—*La infección tuberculosa en las escuelas de Lima, J. E. Tamayo Echevarría, Rev. tuberc., January–June, 1950, 10: 99.*—(*A. A. Moll*)

Tuberculin Sensitivity and BCG.—The sensitivity to tuberculin after administration of BCG is generally regarded as a criterion for successful vaccination. Since the oral use of the vaccine produces only inconstant and transitory skin allergy, the percutaneous methods are preferred at present. The relationship between immunity and allergy, however, is uncertain, and rejection of the safe oral method on this basis is not justified. Tuberculin allergy is a highly specific phenomenon; individuals infected with one type of bacilli react selectively to the tuberculin obtained from that type. The positive reaction obtained with the usual tuberculin after BCG vaccination probably is a group reaction; orally vaccinated individuals react only to tuberculin prepared from BCG. Whether resistance to tuberculosis may be present without positive tuberculin reaction is still a moot question. Children may live for long periods with infectious patients without becoming ill or allergic to tuberculin. A group of 240 children, anergic at the age of eight to twelve years, were followed for fifteen years at the Barcelona Antituberculosis Institute; 48 (20 per cent) fell ill with tuberculosis during the period of observation; none died. In a control group of 1,626 children, already allergic at the same age, 390 cases (24 per cent) of tuberculosis were observed during the same period; 113 (7 per cent) of this group died. This suggests that tuberculin-positive and tuberculin-negative children have approximately the same chance of becoming ill with tuberculosis. If this were confirmed on a larger scale, it might be unnecessary to repeat percutaneous BCG vaccinations to obtain a positive skin test. Oral BCG vaccination has proved adequate to confer a relative immunity in more than 20,000 cases at the Barcelona Antituberculosis Institute.—*Sensibilidad tuberculinica y B. C. G., C. Xalabarder, Rev. españ. de tuberc., August, 1950, No. 185: 535.*—(*L. Molnar*)

Silicosis in Japanese Mines.—The examination of 4,926 workers in metal mines in Japan by direct roentgenography gave the following data, using Oka's classification of increasingly severe silicosis: the total incidence of silicosis was 59.8 per cent; of first-grade silicosis, 46.0 per cent; of second-grade, 8.6 per cent; of third-grade, 2.8 per cent; silicosis combined with pulmonary tuberculosis, 5.5 per cent; pulmonary tuberculosis without silicosis, 2.4 per cent. The incidence rate of silicosis by age groups was: 20 to 29 years, 17.7 per cent; 30 to 39 years, 58.1 per cent; 40 to 49 years, 72.5 per cent; 50 to 59 years, 76.1 per cent; 60 and more years, 77.6 per cent. The youngest worker with first-grade silicosis was 20 years old and the oldest 69; the youngest with second-grade was 30 and the oldest 69; the youngest with third-grade was 35 and the oldest 64. The incidence rate of each grade of silicosis when plotted on probability paper in relation to age shows a normal distribution for all groups 30 years old or older. Likewise, the incidence rate of each grade shows a normal distribution in relation to the length of service in dusty occupations. The incidence of pulmonary tuberculosis, including healed pleurisy and primary tuberculosis, was 28.7 per cent in miners without silicosis, 34.8 per cent in miners with first-grade silicosis, 47.8 per cent in those with second-grade, and 54.0 per cent in those with third-grade silicosis. The incidence of progressive tuberculosis was 6.9 per cent in nonsilicotic miners, 4.9 per cent in miners with first-grade, 16.5 per cent in those with second-grade, and 36.3 per cent in those with third-grade silicosis. The incidence of pulmonary emphysema was higher in silicotic than in nonsilicotic miners, especially in the younger age groups. Emphysema, however, increases steadily with age in both groups and appears to be a function of age rather than a complication of silicosis. Vital capacity, both in normal and in silicotic miners, was found to decrease consistently with age. In workers with first-grade silicosis the vital capacity was not lower than in normal workers of the same age but in those with third-grade silicosis it was 10 per cent lower in each age group.—*Report on a country-wide study of silicosis in the metal mines of Japan, M. Yamamoto, Arch. Indust. Hyg. & Occup. Med., April, 1951, 3: 339.*—(*T. H. Noehren*)

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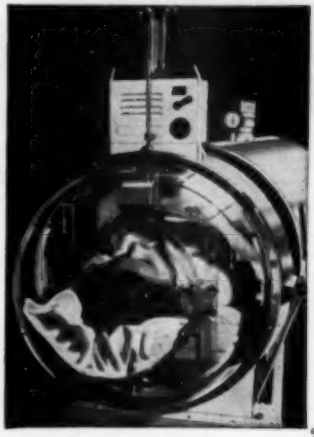

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*McLester, J. S.: Nutrition and Diet in Health and Disease, ed. 5, Philadelphia, W. B. Saunders Company, 1949, p. 588.

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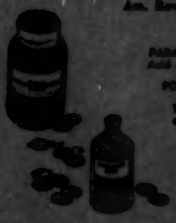
TEMPEL, C. W., FEERICK, J. H. Jr., HARDIN, R. C., TOWERS, H. L., and BYE, W. E. Combined [illegible] experience employing streptomycin and PAS. Am. Rev. Tuberc., 1952, 65, 205



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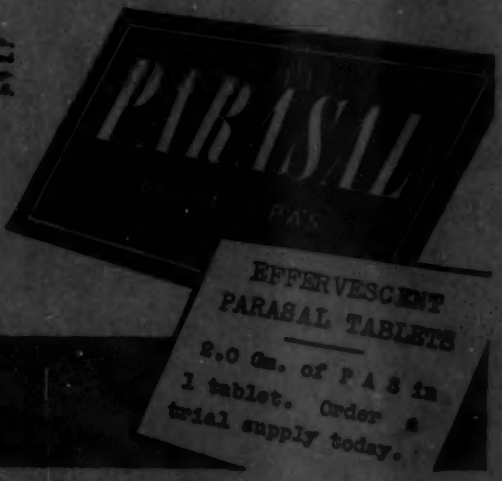

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